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Microctonus vittatae, a Parasite of Adult Flea Beetles, and Observations on Hosts

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The striped flea beetle, *Phyllotreta striolata* (F.), a widely distributed pest of cultivated Cruciferae in the holarctic realm, was introduced into America from Eurasia before 1801. This species received considerable attention in the early entomological literature of the United States, chiefly due to the fact that the larvae and adults produced extensive damage. Except in the South where the variety *discedens* Weise is the dominant form, reference to it is infrequent in the more recent literature. Chittenden (1927) refers to it as a "pernicious species" which "in some years severely injures cole crops, destroying entire plantings, and damage is particularly severe in seed beds."

Phyllotreta striolata was first recorded as a host of a braconid parasite in 1906. Chittenden & Marsh (1920) stated that this parasite is sometimes an important enemy of the "striped cabbage flea-beetle, *Phyllotreta vittata* Fab." This braconid was named *Microctonus vittatae* by Muesebeck (1936) after Peterson had furnished a large series of adult females during the initial phase of this investigation in 1932. The results reported in this paper come from experiments conducted during 1932, 1948 and 1949, designed for study of the incidence, seasonal abundance, distribution, and biology of *M. vittatae*. More detailed findings on the biology of this parasite will be reported in a separate paper.

METHODS AND MATERIALS.—Dissection of field collected beetles proved to be the most reliable method for the determination of the percentage of parasitism. Another method was tested, namely that of making counts of parasites which emerged from confined beetles. Table 1 shows the distinct differences obtained from the two methods. The differences can be accounted for largely by the high mortality of confined beetles from undetermined causes.

Such mortality for the confinement period (approximately 2 weeks) ran 42.4, 42.8, and 34.49 per cent respectively for the three groups of beetles confined. If these percentages are added to the per cent parasitism determined by the emergence method, the figures obtained are near those of the dissection method. Totaled figures and per cent parasitism by dissection compare as follows: 59.3 to 75.0; 52.02 to 66.66; and 57.42 to 42.8 per cent respectively.

During 1932 approximately 50 beetles were collected at irregular intervals by means of an aspirator and dissected alive within 24 hours in 70 per cent alcohol. During 1948 and 1949 the beetles were collected at weekly intervals (on the same dates of each year) and dissected alive in DeFruze Ringer's saline solution. By this method parasites and beetles remained alive for a period of an hour or more. The number of beetles dissected at one time was never less than 25. Percentages of parasitism based on this figure were

Table 1.—Comparative incidence of parasitism determined by dissection and emergence methods.

Dissection Method			Emergence Method		
Date Coll.	No. Beetles Dissected	Per Cent Parasitism	Per Cent Parasitism	No. Beetles Confined	Date Coll.
3/28/49	20[1]	75.0	17.1	420[1]	3/28/49
7/16/49	27	66.66	9.22	1,500	7/14/49
8/ 6/49	28	42.8	22.93	1,212	8/ 4/49

[1] Overwintering beetles.

[1] The junior author investigated the biology of this parasite during 1932 when he was employed by the Bureau of Entomology and Plant Quarantine under N. F. Howard at Columbus, Ohio.

almost identical to those based on 50 beetles.

Distributional data in North America were obtained by dissection of preserved *Phyllotreta striolata* which had been collected by cooperating entomologists at 17 different experiment stations in the United States and Canada. Identification of the parasite was made solely upon larval characteristics which had been worked out carefully during the biological phase of this investigation.

Observations on the winter mortality of the striped flea beetle were made incidental to the study of the overwintering form of the parasite. Exactly 1000 beetles were confined in the fall in each of two cages set on a more or less protected south slope and survival counts were made the following spring. Two other cages were placed in an exposed garden area but the number of beetles confined in these was not accurately determined. The hibernation cages had open bottoms and measured 25 by 15 by 22 inches. They were covered with organdy and reinforced with copper screen. Their fronts were furnished with a muslin sleeve, all cracks were sealed with beeswax, and their bottoms were fitted with a sheet metal flange. The cages were driven into the ground to a depth of five inches over and about growing turnip and horseradish and were supplied with hibernation litter, especially sections of cedar posts with deep cracks, small limbs with loose bark, leaves, etc.

OUTLINE OF LIFE-HISTORY OF *Microctonus vittatae*.—The biology of the parasite is discussed fully in a separate paper. A brief account of the bionomics of this insect is included here. *M. vittatae* is a thelyotokous species, males being exceedingly rare. Fertile eggs may be oviposited within a few minutes after emergence of the wasp from the cocoon. Diminutive eggs oviposited in the thoracic regions of the beetles increase in volume by approximately 1200 times during incubation. At the time of eclosion, the trophamnion surrounding the embryo is broken down into component cells which thereafter float free in the host fluids, continuing their trophic function of absorbing fats, ultimately attaining gigantic proportions (a volumetric increase in size of 3500 times). These "giant cells" are the chief source of food for the parasite larvae in their final stages. They are excellent indicators of parasitism and have frequently been mistaken for disorganized fat-bodies of the host. Only one larva develops per host although superparasitism occurs. The supernumeraries are eliminated at an early stage by cannibalism or by "enzymatic" secretions of their rivals. During the course of larval feeding the adipose tissue of the host is destroyed and female beetles are castrated. Full grown larvae force their way out of the caudal end of their hosts, invariably killing the beetle. Shortly thereafter they spin white silken cocoons. The duration of the cocoon period varies from 7 to 20 days depending upon the temperature. The number of larval instars was not determined for certain, apparently there are 4 or 5. At 70° F. the entire life history may be completed in 18 to 20 days. This may be broken down further as follows: egg stage 5 to 6 days; first instar 4 days; and 1 day for each succeeding instar. The first instar is the normal overwintering form and its duration is variable depending upon the season of the year. At Columbus, Ohio it is estimated that there are 6 to 7 generations of the parasite per annum compared with one for the host species.

HOSTS OF *Microctonus vittatae*.—This braconid has been reared from the following vittate Phyllotreta: *P. striolata* (F.), *P. striolata discedens* Weise, *P. striolata lineolata* Chitt., and *P. bipustulata* (F.). Parasite larvae, which were indistinguishable from *M. vittatae* were found in *P. zimmermanni* (Crotch). *P. aerea* Allard, a unicolorous species apparently is never parasitized in the field, however it was parasitized in the laboratory. Whether the parasite can complete its development in this species or not is unknown. *P. bipustulata* and *P. zimmermanni* appear to be native hosts of this parasite and from these indigenous hosts it transferred to the imported *P. striolata*.

DISTRIBUTION OF *Microctonus vittatae*.—The locations from which adult *Phyllotreta striolata* containing *Microctonus vittatae* larvae were collected together with localities in which the parasite has been reared are shown by the bold dots in figure 1. The stippled areas indicate the known distribution of the hosts of this parasite in the United States and southern Canada. Also *M. vittatae* has been reported from Japan, but since this identi-

fication was made solely from the author's description of the imago, Muesebeck[1] is not certain that this determination is correct. Unfortunately, the southern states are not well represented in this survey due in part to sending the request for beetles after the cole crops had been harvested. None of the 53 beetles (mostly *Phyllotreta discedens* Weise) dissected from Louisiana contained parasites which seems to confirm the statement of Dugas (1938) that *M. vittatae* is not numerous in that state. In spite of the greater difficulty of locating parasites in preserved specimens, *M. vittatae* larvae were found to occur in all other states and provinces from which *P. striolata* was received. Its range thus appears to coincide with that of the striped flea beetle, namely east of the Rocky Mountains, however, it appears to be less abundant in the southern states.

INCIDENCE OF PARASITISM AND SEASONAL ABUNDANCE.—The incidence of parasitism was determined only for *Phyllotrata striolata* at Columbus. The results are presented graphically in figure 2. The plotted lines are an average of three observations on 25 or more field collected beetles. The faint portion of the 1932 curve represents a period when no observations were made. Unfortunately the record for none of the years is entirely complete nor do the observations cover identical periods for all three years. Disregarding for a moment the sharp drop in parasitism in late July and early August 1932 together with weekly fluctuations, it will be noted that parasitism followed similar trends during the three seasons. In general, it reached a peak toward the end of July and thereafter more or less gradually tapered off until the end of September and again rose abruptly in the late fall. Parasitism of hibernating beetles taken from either natural sites or hibernation cages ran high during the winter of 1948–49, however, insufficient beetles were found at any one time for making accurate estimates of percentages of parasitism. This was also true of beetles taken early in the spring. Extremes in variation of parasitism at different times in 1932 were from 12 to 80 per cent; in 1948 from 20 to 91.7 per cent; and in 1949 from 21.4 to 56.7 per cent.

The average percentages of parasitism for the three years are shown in table 2. It will be noted that the seasonal abundance of the parasite is consistently high. In 1932, 45 per cent of the striped flea beetles were parasitized; 53.6 per cent in 1948; and 40.8 per cent in 1949; or an average of 46.37 per cent for the three years. The totals for 1948–49 show that the sexes were affected about equally in proportion to their ration. What causes the fluctuations noted has not been determined. The lows in parasitism recorded for September apparently are correlated with the heavy emergence of young beetles during that month. The relatively low parasitism during 1949 appears to be a result of low host populations. Although no population studies of the flea beetle were made during 1948, 50 beetles could be collected each week with ease while in 1949 it was often difficult to obtain 25 beetles for the weekly dissections. *M. vittatae* was found to be very sensitive to moisture relations, especially at the time of cocoon spinning and emergence from the cocoon. Excess moisture or dryness resulted in the death of the parasite. Periods of drought or heavy rainfall could in large measure account for the weekly and annual fluctuations in parasitism.

Table 2.—Seasonal abundance of *Microctonus vittatae* over a 3-year period.

	Beetles Dissected			Per Cent Beetles Parasitized		
Year	Total	Per cent Male	Per cent Female	Total	Per cent Male	Per cent Female
1932	1050	—	—	45.00	—	—
1948	640	57.20	42.80	53.60	59.30	46.00
1949	514	58.37	41.63	40.80	44.00	34.58
Totals 1948–49	1154	57.71	42.29	47.58	52.40	44.60
Totals 3 years	2204	—	—	46.37	—	—

Microctonus vittatae AS A CONTROLLING FACTOR.—This parasite appears to be of distinct importance and probably is the key agent in the control of *Phyllotreta striolata* in the United States north of Mason-Dixon and in southern Canada. The percentage of flea beetles destroyed by adult mortality during two consecutive years and one widely separated year was consistently high. Since castration of females results from parasitism, hundreds of potential flea beetles were destroyed prematurely in the 42 per cent of the females that were parasitized. If sterility is produced in hibernating beetles, which

[1] (Correspondence 1949.)

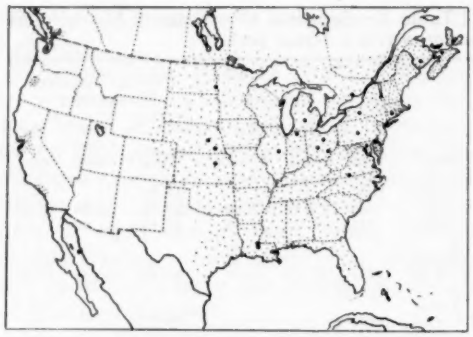

FIG. 1—Distribution of *Microctonus vittatae* in the United States.

is true in certain other *Phyllotreta* parasitized by other species of *Microctonus* (Kurdiumov & Znamenski 1917), the effectiveness of *M. vittatae* is greatly enhanced because the per cent of hosts parasitized is very high from late fall to early spring. The presence of *M. vittatae* also seems to account in large measure for the fact that neither *P. bipustulata* nor *P. zimmermanni* are pests of very great economic importance.

The differences in the opinions of the early economic entomologists in northern United States (Shimer 1869, Riley 1884) compared with those of the present day (Frost 1949, Sleesman 1950), with respect to the importance of *P. striolata* as a pest, may in part be explained by the transfer of this parasite from its native hosts to this imported flea beetle. The fact that the variety *discedens* is still considered highly injurious in the south (Dugas 1938) seems to be correlated with the scarcity of the parasite in the gulf states. The additional fact that the striped flea beetle is considered a major pest in the Orient (Harukawa & Tokunaga 1938, Liu and Yen 1941) appears to be related to the absence of *M. vittatae*. It thus appears that this braconid is worthy of further study. Its introduction into regions of the world where *P. striolata* remains a serious problem might prove to be distinctly beneficial. The fact that it spends several months within its hosts at a time when the latter are young and vigorous, makes it particularly adaptable to long shipment in refrigeration.

Hyperparasites of this species are unknown. Probably it is attacked by predators during its free existence, especially while it is in the soil during its last larval and pupal stages.

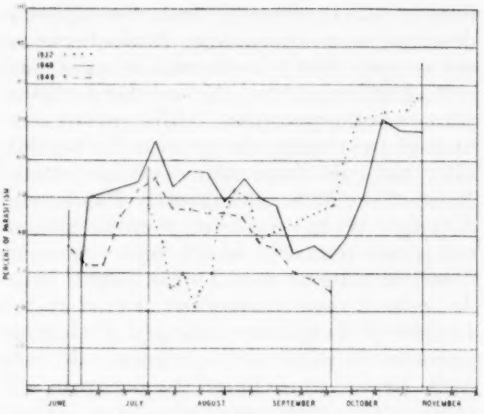

FIG. 2—Three-year record of parasitism.

HIBERNATION AND OTHER PARASITES OF HOSTS.—Harukawa & Tokunaga (1939) made hibernation studies of *Phyllotreta striolata* in Japan and Harukawa & Nisikawa (1941) investigated the resistance of this species to low winter temperatures. They found that the adult stage only survived the winter in the field with temporary suspension of activity but no true diapause. Also winter survival is low with little or no difference between sexes. Results at Columbus during the mild winter of 1948–49 give similar indications although insufficient replicates were run for accurate conclusions. Recovery was obtained in all four field cages. In the two cages in which the number of beetles was known, survivals of 20.6 and 21.8 or an average of 21.2 per cent were obtained.

The sex ratio of *Phyllotreta striolata* was determined by keeping record of the sexes during the weekly dissections scattered over two years. An average of 57.71 per cent of the 1154 beetles dissected were males. This compares with a ratio of 53.7 per cent males for 3625 field collected beetles in Japan (Harukawa & Tokunaga 1938).

An allantonematid parasite, indistinguishable from *Howardula phyllotratae* Oldham, was found to infest *Phyllotrata striolata* and *P. bipustulata*. The larvae of these species develop in subterranean root systems. Adult females of this nema overwinter in the body cavity of *P. striolata*. Hundreds of larval nemas were found also on occasion within the hosts in the late summer, and in *P. bipustulata*, taken at Manhattan, Kansas. At Columbus it was present, however, in insigni-

ficant number during 1948 and 1949, 7.6 and 2.9 per cent respectively of the beetles dissected for the two years being parasitized. It was not found in *P. zimmermanni*, whose larvae mine the leaves of host plants. The larvae of the latter species are attacked by a chalcid, *Pleurotropis phyllotretae* Riley. Heikertinger & Csiki (1939–40 Junk Catalogue) list this same parasite for *P. striolata* giving as authority Elliot & Morley (1911). This is a misquotation. The larvae of *P. striolata* were not studied consequently it is unknown whether or not they are parasitized by nemas.

An acarinid (Trombidiidae), in its larval stage, preys upon adults of *Phyllotreta striolata*, *P. bipustulata* and *P. aerea* during June and July. As many as 15 mites were found under the elytra of a single beetle, with their hypostomes inserted deeply into the intersegmental areas of the thorax and abdomen. During the latter part of June 1948 they were quite abundant on *P. striolata*, 51.0 per cent of the beetles being infested. For the year 1948, however, only 8.7 per cent of the beetles examined bore mites and in 1949, 2.1 per cent. No deleterious effects of their feeding were noted.

SUMMARY.—A three year study (1932, 1948, and 1949) of *Microctonus vittatae* Mues., a braconid parasite of adult flea beetles, reveals that this parasite is probably the key agent which keeps its hosts, *Phyllotreta striolata* (F.), *P. bipustulata* (F.), and *P. zimmermanni* (Crotch), in check in northern United States and southern Canada. Incidence of parasitism of the striped flea beetle ran 45.00, 53.60, and 40.80 per cent respectively, or an average of 46.37 per cent for the 3 years. In addition to adult mortality at the time of emergence of the parasite larvae female beetles are castrated before they are killed which greatly enhances the efficiency of the braconid. Nametodes and Acarina also infest adult flea beetles to a limited extent. Further study of *M. vittatae* is needed, also it might prove to be highly beneficial if introduced into regions of the world where the striped flea beetle is still a serious pest of cruciferous crops in gardens.

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A Large-Scale Test with DDT to Control the Japanese Beetle

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To determine the degree of control of the Japanese beetle, *Popillia japonica* Newm., that could be obtained with general applications of DDT to the soil and to favored food plants in a well-established, isolated infestation, a large-scale test[2] was carried on at Blowing Rock, N. C., during the period 1945 through 1948. The results of this test, which may be of interest to regulatory and control officials or to civic groups that may be contemplating community chemical control action against the beetle, are presented in this report.

Although the original plans for the test included yearly foliage treatments for favored food plants during the period of the adult beetle activity, to supplement the soil applications of DDT, this phase of the work was restricted to a few applications of limited control value.

Physical Features of Blowing Rock.—Blowing Rock is a resort town at an elevation of 3,600 feet in Watauga County, in northwestern North Carolina. The terrain is hilly and most of it is heavily wooded, with evergreens predominating. Part of the land has been cleared. There are several hotels, an 18-hole golf course, many residences with well-kept lawns, many small farms, and a few large estates. Fields, pastures, and orchards are scattered throughout the area.

Climate.—The climate at Blowing Rock is quite different from that in the central and eastern parts of the State, and is similar to that in central New England. The temperature is relatively low during the summer months, and freezing frequently occurs between October and April. Rainfall is often heavy during the summer, ranging during this test from 14 to 22 inches. Fog is common, even in midsummer.

The Soil.—The soil at Blowing Rock has been classified as Ashe loam.[3] It is derived from underlying rocks, including granite, gneiss, and dark-colored basic rocks, such as diorite and hornblend schist. The rather steep relief has retarded the formation of a normally mature soil, and in some places erosion has kept close pace with the soil-forming processes. Outcrops of rock are common. On the steeper slopes there are rather extensive areas of rock outcrop or very shallow soil. Leaching of the soluble materials has been active and is still in progress. The heavy rainfall and the moderate temperature allow leaching during the greater part of the year.

Ashe loam is not very important agriculturally, most of it being in forests or pastures, but some crops including corn, potatoes, cabbage, and small grains are grown on the smoother areas and there are a few scattered apple orchards. No complete chemical analysis of the soil has been made. Partial analyses made of the Ashe series[4] in Georgia and in Virginia indicate that the soils of this series are well supplied with potash, but tend to be deficient in phosphoric acid, nitrogen, and lime.

History of the Infestation Prior to 1945.—The presence of the Japanese beetle at Blowing Rock was established definitely during the summer of 1943 when 17,045 beetles were captured in a few traps. The beetle had evidently been there for several years, but its presence had not been discovered. Nothing definite is known about its introduction into this isolated section.

In 1944 approximately 500 traps were placed throughout the town and the surrounding territory to obtain more definite information on the infestation. In all, 138,945 beetles were captured, indicating a well-established infestation over at least 250 acres. The beetles were most numerous in the northeastern section of the town, and were relatively scarce in the other sections of the town and in the surrounding territory.

Seasonal Cycle of the Japanese Beetle.—The seasonal cycle of the beetle

[1] R. D. Chisholm analyzed the different batches during the processing and formulation of the 10 per cent DDT, and also analyzed samples of soil to determine the concentration of lead arsenate and DDT.

[2] In cooperation with the North Carolina Department of Agriculture.

[3] Davis, W. A., Cobb, W. B., and Devereux, R. E. Soil Survey of Watauga County, N. C. Bur. Chem. and Soils, U. S. Dept. Agr. S. S. Rpt., 1928, No. 1, 1930.

[4] Joffe, J. S., and Conybeare, A. B. Analyses of United States Soils. Section 2: Southern Atlantic States. N. J. Agr. Expt. Sta. April 1943.

at Blowing Rock approaches its usual cycle in central New England. During each of the four years of this test, the first beetle was found late in June, the peak of abundance occurred about the middle of August, and beetles were still numerous during September. Oviposition continued as long as beetles were present. Some of the eggs were probably killed by cold weather. Second instars made up from one-third to four-fifths of the normal overwintering soil population and a few first instars were always present.

FOOD PLANTS AND FEEDING HABITS OF THE JAPANESE BEETLE.—Japanese beetle adults showed the same food preferences at Blowing Rock as in other areas, but many of the preferred plants are not available there. Most of the trees are evergreens on which the beetle does not feed, and many of the deciduous trees are not favored food plants. Feeding was commonest on low-growing hosts such as berries, grape, woodbine, evening primrose, and elder, with occasional feeding on smartweed, apple, rose, and soybean. As the summer climate is cool and humid, the beetles tend to rest and feed on the low-growing plants and do little flying.

SOIL TREATMENTS PRIOR TO 1945.—Late in the fall of 1943 about 25 acres of lawns in the known infested area were treated with lead arsenate at the rate of 500 pounds per acre. Chemical analyses of the turf 2 years later indicated that none of the arsenical had been lost. About 90 per cent of it was still within 1.5 inches of the surface, but its distribution was very irregular, ranging at different points from 74 to 1151 pounds per acre. The lack of uniformity in lateral distribution is believed to be due, in part, to the irregular topography.

As it was considered undesirable to apply lead arsenate to pastures and vegetable gardens, about 40 additional acres of pasture land and gardens were treated with type A milky disease in the fall of 1943. The spore dust was applied as a spot treatment at 5-foot intervals.

After the summer of 1944 it was evident that the lead arsenate and the spore dust treatments made in 1943 to about 25 per cent of the area known to be infested in 1944 had had only a nominal effect on the further development of the infestation.

SOIL TREATMENT WITH DDT.—From May 22 to June 23, 1945, DDT was applied to practically all the known infested soil in the Blowing Rock area, except the 25 acres that had been treated with lead arsenate in the fall of 1943, including the golf course, lawns, pastures, fields, vegetable gardens, flower beds, and shrubbery borders. Applications were not made in wooded areas where there was no turf, in low swampy areas where it was impossible to use the equipment, or on steep slopes that were practically devoid of soil. In the fall of 1945 DDT was applied to the 25 acres previously treated with lead arsenate and 9 additional acres, making a total of 251.8 acres treated in 1945. A final soil application of DDT was made in 1947 when 5.2 additional acres found infested in 1946 were treated. The entire area treated with DDT contained approximately 257 acres.

The DDT used was of the technical grade, and was a powder of such fineness and flowability that not less than 9 per cent passed through a 1 mm. sieve (U. S. Standard No. 18) by dry test. The DDT was processed by micronizing it with an equal weight of pyrophyllite in 1000-pound batches. After the mixture was ground, chemical analysis showed that the DDT content was 49.96 per cent. The processed material was of such fineness that 90 per cent of the particles did not exceed 10 microns in longest dimension, and 99.9 per cent did not exceed 20 microns. The 50-per cent micronized DDT was mixed in 700-pound batches with additional pyrophyllite and tricalcium phosphate of the conditioner grade so that the final product of 65,000 pounds contained 10 ± 0.5 per cent of DDT, 87 per cent of pyrophyllite, and 3 per cent of tricalcium phosphate. It was packaged in 50-pound units for ease of handling in the field.

The 10 per cent DDT dust was applied to the soil in large areas with a 10-foot tractor-drawn spreader and to small areas with 3-foot hand-drawn spreaders, set to deliver 250 pounds per acre. The delivery of the dust was relatively constant from day to day, regardless of weather conditions. For the 10-foot spreader set to deliver 250 pounds per acre, the standard deviation was 12.9 pounds, or 5.21 per cent, and for the 3-foot machines the standard deviation was 13.5 pounds per acre, or 5.41 per cent.

The standard deviation for the entire spreader operation was 13.11 pounds per acre, or 5.21 per cent. It may be concluded, therefore, that each acre treated with a spreader received 25 ± 1.3 pounds of DDT. On extremely rough terrain, vegetable gardens, flower beds and shrubbery borders the DDT dust was broadcast by hand.

A total of 963 man-hours was required to apply 62,950 pounds of 10 per cent DDT to 251.8 acres. The average time was 30.6 man-hours per ton of dust, or 3.8 man-hours per acre, but this varied considerably with the method employed and the nature of the area.

The authors obtained a sample of Ashe loam from Blowing Rock in the fall of 1944 for preliminary biological tests before undertaking the large-scale treatment with DDT. After removing stones and debris, DDT was thoroughly mixed with the soil at the rate of 25 pounds per acre of soil to a depth of 3 inches, or 10,890 cubic feet. At intervals after the application of DDT, 150 third-instar larvae were introduced into the treated soil and the time required to kill 98 per cent of each batch was determined. The average time required was 3 weeks. The velocity of insecticidal action in Ashe loam was about the same as in soils of similar texture from New Jersey and New England. In a series of 15 tests extending over a period of 2 years, there was nothing to indicate that the velocity of insecticidal action was retarded abnormally in Ashe loam or that the effectiveness of the treatment had been modified significantly. It was evident that the soil at Blowing Rock was suitable for treatment with DDT.

Analyses of soil samples taken at Blowing Rock during a period of 2 years following the application of DDT indicated that no significant amounts of DDT had been lost and that very little of the chemical had penetrated beyond a 1.5 inch depth.

FOLIAGE APPLICATIONS OF DDT.—In the summer of 1945 a total of 7950 gallons of spray containing 2 pounds of 50 per cent micronized wettable DDT per 100 gallons was applied to deciduous trees, shrubs, and vines, along the roads and on different properties within the infested area. About 150 pounds of 10 per cent DDT dust were also applied to grapevines and other low-growing plants that could not be reached conveniently with the spray, or were found to be infested later on in the summer. Since the beetles under the conditions prevailing at Blowing Rock seldom visited the tall deciduous trees and shrubs, feeding instead on low-growing plants, probably only limited benefits resulted from the spray application. In the summer of 1946 foliage treatments were restricted to the application of 125 pounds of 10 per cent DDT dust to low-growing food plants at 29 locations. Although the foliage treatments undoubtedly killed some beetles, it is believed that only a comparatively small proportion of them was destroyed, and that the effect of the foliage treatments on the final results was negligible.

RESULTS.—The effectiveness of the DDT treatments in controlling the Japanese beetle at Blowing Rock was determined by making grub surveys in a selected plot at various intervals after treatment, and by using traps to capture beetles that emerged from the treated areas during subsequent summers.

GRUB SURVEYS.—A 2-acre pasture near the center of the infestation was selected to study the effect of the treatment on the immature stages of the Japanese beetle in the soil. The treatment had a greater effect on the grubs than was expected. Many of them were full grown and had stopped feeding, and were therefore not susceptible to the poison. In spite of this factor, the number of grubs or pupae per square foot dropped from 6.6 the day before the treatment was applied, to 1.9 five weeks later. Early in August 1945, before the treatment began to affect the young grubs, the average grub density of the 1945–46 brood was 29.9 per square foot. A year later the density of the 1946-47 brood was only 6.3 per square foot, indicating that 78.9 per cent fewer eggs had been deposited in the soil in 1946 than in 1945. The DDT treatment in the spring of 1945 eliminated about 75 per cent of the 1945–46 brood by October in the same year and about 90 per cent by the following June. No untreated turf was available near by to afford information on the normal populations, but presumably the numbers would have increased if the poison had first been applied. Unfortunately it was necessary to abandon this phase of study in the spring of 1947, when the pasture field was re-

graded and converted into a riding track.

At Blowing Rock, as indicated above, a reduction of 90 per cent in the 1945–46 and the 1946–47 broods was not obtained until the spring in which the grubs completed their development. In New Jersey, Connecticut, and Massachusetts reductions to this extent are usually obtained by September of the year in which the eggs are laid. The lower rate of insecticidal action at Blowing Rock can be attributed to the slower development, the lower activity of the grubs, and differences in their feeding habits caused by the cool weather which prevails during the summer. Under these conditions the larvae are less active in feeding and moving through the soil and a longer period is required for them to obtain a lethal amount of DDT. Furthermore, in contrast with the situation at the other locations, many were found below the poisoned soil layer, where there is less opportunity for them to encounter particles of DDT.

ADULT-BEETLE SURVEYS.—Although grub-population studies in the small pasture showed that the DDT treatment had reduced the population materially, adult-beetle surveys proved to be a more practical method of appraising the value of the treatments over the whole area.

During the summers of 1944, 1945, 1946, 1947, and 1948 approximately 600 Japanese beetle traps were operated at Blowing Rock. These were baited with a mixture containing 90 per cent by volume of technical anethole and 10 per cent of U. S. P. eugenol. About nine-tenths of the traps were placed within the treated area and the remainder in the adjacent untreated area. With a few minor exceptions, the traps were placed in the same positions each year. They were placed when the beetles began to appear and were not removed until beetle activity had ceased in the fall. At weekly intervals the beetles captured by each trap were removed and counted.

In the untreated area at Blowing Rock the numbers of adult beetles captured by the traps increased rapidly, from 357 in 1945 to 2612 in 1946, to 7727 in 1947, and to 16,798 in 1948; or, in comparison with the 1945 catch, a 6.3-fold increase by 1946, as 20.6-fold increase by 1947, and a 46.1-fold increase by 1948. From this trend, it is estimated that, had no DDT been applied, the number of beetles captured in the treated area would have increased from 138,945 in 1944 to 1,014,298 in 1945, to 3,001,212 in 1946, to 6,544,309 in 1947, and to 9,865,095 in 1948.

In the treated area the situation was entirely different from the theoretical picture. The total number of beetles captured in the traps declined from 138,945 in 1944 to 41,914 in 1945, to 19,623 in 1946, to 23,361 in 1947, and to 15,908 in 1948, or reductions amounting to 70 per cent of the 1944 catch in 1945, 86 per cent in 1946, 83 per cent in 1947, and 89 per cent in 1948.

EFFECT OF DDT ON OTHER INSECTS.—Some observations were made on the effect on other insects of the DDT treatment of soil. Within a few hours the DDT dust killed most of the insects that came in contact with it above ground. Observations in subsequent years indicated that ground beetles were practically eliminated, but that most of the other species have re-established themselves. The soil treatment practically eliminated cutworms, but seemed to have little permanent effect on scarabaeid grubs other than the Japanese beetle, or on wireworms, staphylinids, weevils, and ants, possibly because most of these inhabit the soil below the poisoned layer. Even though flies are usually abundant at Blowing Rock because of the large number of cows and horses in the pastures throughout the year, there were very few during 1945 and 1946. The fly population showed a definite increase in 1947.

EFFECT OF DDT ON OTHER ANIMALS.—Many sowbugs were killed by the soil treatment but other invertebrates, such as earthworms, garden slugs, millepedes, and centipedes did not seem to be affected. Soon after the DDT was applied a few dead and dying birds were found, including robins, brown thrashers, catbirds, blackbirds, and sparrows, and several nests were found with unhatched eggs or dead fledglings. From all indications, the deaths were the result of poisoning from eating DDT-contaminated insects. The effect on birds seemed to occur during a relatively short period following the application of the dust to the surface of the soil. A year after treatment the different species were apparently as numerous as ever. There was no evidence to indicate

that DDT reduced the number or extent of mole runways, which were common at Blowing Rock. For a short time after the treatment a few dead and dying toads were found.

Most of the streams and ponds in the treated area are heavily stocked with fish. The turf along some of the streams and ponds was treated, and it is possible that some of the DDT was washed into the streams and ponds during heavy rains. Although several examinations were made, there was no evidence of appreciable or permanent injury to fishes, frogs, or other aquatic life.

As a precautionary measure, it was requested that all domestic animals be kept out of fields and pastures dusted with DDT until the material had been washed into the soil by rain. In many localities the people did not comply with this request. Flocks of chickens that were allowed to run in treated fields and pastures immediately after the dust was applied did not appear to be harmed. Cows and horses were pastured in treated areas after the first rain, but no abnormal animals were observed and there were no reports of sick cows or horses. Cats and dogs, often permitted in the treated areas before the dust had been washed into the ground, apparently were unaffected.

CONCLUSIONS.—The application of DDT to the soil at the rate of 25 pounds per acre, in a large-scale test at Blowing Rock, N. C., in 1945, and limited foliage applications to kill adult beetles in 1945 and 1946, prevented normal increases in the population density of the Japanese beetle and by 1946 had reduced the population to a relatively low level. As a result of the residual effectiveness of the DDT in the soil, the population in the treated area remained at this low level through 1948, and there is every indication that there will be no significant increase for several years. In contrast, the beetle population increased rapidly in the surrounding untreated area.

The treatments were not sufficiently inclusive to provide definite information on their value for eliminating isolated Japanese beetle infestations. However, observations suggested that eradication would not be possible in any event without having a continuing program for re-treating areas where the soil layer containing DDT has been removed or made ineffective by plowing, digging, landscaping, and other operations, and for treating additional small local infestations that may develop outside the treated areas. Unless control measures are applied to infestations outside the treated area, it may be expected that such new infestations will continue to develop and normal spread of the beetle to the surrounding countryside will continue.

The major objective of this large-scale test was accomplished—to demonstrate the value of general applications of DDT in suppressing an isolated infestation.

Dieldrin for Cotton Insect Control[1]

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A new synthetic insecticide, deildrin, was tested against eight species of cotton insects and a spider mite at College Station, Texas, in 1949. The tests were designed to establish the fact of toxicity and to determine the dosage required for effective control of the various insects.

Formulations received from the manufacturer were a 15-per cent dust concentrate, a 25 per cent wettable powder, a 25 per cent emulsifiable concentrate, and the technical material. Dust concentrations of less than 25 per cent were made by diluting the concentrate or wettable powder with pyrophyllite. A 40 per cent concentration used in one test was prepared by first grinding the technical material, a crystalline solid, in a mortar and then blending it with pyrophyllite in a ball mill. Spray formulations were prepared from the technical material with acetone as a solvent and polyethylene glycol phenylisooctyl ether as an emulsifier, and from the 25 per cent emulsifiable concentrate.

Tests were conducted both in field cages

[1] Report of a study made under the Research and Marketing Act of 1946, in cooperation with the Texas Agricultural Experiment Station.

[2] Presented at the meeting of the Texas Entomological Society, Houston, Texas, January 19–20, 1950.

and in the laboratory. The procedure, described by Ivy (1944), of placing a settling chamber over plants to be treated was used in the field-cage tests. After the treatment the chamber was removed, cages were placed over the plants, and the insects released. Comparable untreated check cages were used in all tests and the net mortality was calculated by Abbott's formula. In the laboratory tests a dusting tower was used to treat leaves, plants, or portions of plants in the same manner as described for the field-cage tests.

The various tests conducted against the different insects are discussed briefly in the following paragraphs. The results of the dusts tests are summarized in table 1. Dosage-mortality curves for six of the insect species are given in figure 1.

BOLL WEEVIL, *Anthonomus grandis* Boh.—A dust containing 5 per cent of dieldrin gave a higher kill of weevils and one containing 2.5 per cent, a slightly lower kill than the 20 per cent toxaphene dust. Statistically, there was no significant difference in the effectiveness of these three formulations. However, a dust containing 1.25 per cent of dieldrin gave a significantly lower kill.

Sprays were applied at the rate of 3 gallons per acre. Sprays prepared from the technical dieldrin tended to clog the spray nozzle, and were not so effective as the dust formulations. Sprays prepared from a 25 per cent emulsifiable concentrate were as effective as dusts.

Dieldrin in both spray and dust formulations appears to be somewhat more resistant to removal by rain than does toxaphene. Therefore, special tests were conducted to determine the resistance of these materials to showers. After being treated with insecticides the plants were sprayed with water in a portable shower stall. Water from a spray nozzle passed through a 16-mesh screen before hitting the plants, giving the effect of a driving rain. One quarter-inch of simulated rainfall was used in all the tests. After the plants had been washed, insects were caged on them, as in the other tests.

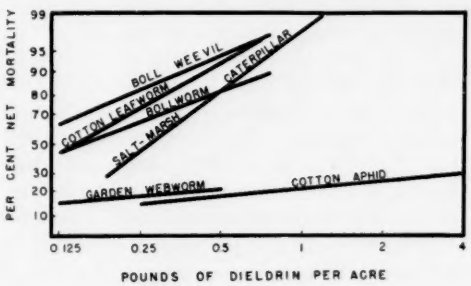

FIG. 1—Mortality of several cotton insects caused by various dosages of dieldrin.

After the artificial shower, sprays or dusts at 0.25 and 0.5 pound of dieldrin per acre were more effective than toxaphene at 2 pounds per acre. The addition of either of two commercial stickers applied at the rate of 0.5 pound per acre did not increase the effectiveness of the 0.25-pound treatments on washed plants.

Table 1.—Per cent mortality of several cotton pests fed foliage dusted with dieldrin or other insecticides. All dusts applied at the rate of 10 pounds per acre unless otherwise specified.

Treatment (Per Cent of Active Ingredient)	Field Cage Tests—Mortality After 5 Days					Laboratory Tests—Mortality After Indicated Period			
	Boll Weevil	Boll-worm	Tobacco Bud-worm	Cotton Leaf-worm	Garden Web-worm	Salt-marsh Cater-pillar[1]	Varie-gated Cut-worm[1]	Cotton Aphid	Spider Mite
						10 Days	*5 Days*	*1 Day*	*2 Days*
Dieldrin									
40	—	—	—	—	—	—	—	29	—
20	—	—	—	—	—	100	—	16	—
10	—	—	—	—	—	99	—	17	—
5	95	85	92	95	21	98	—	21	—
2.5	81	57	—	65	19	51	100	15	8
1.25	68	47	—	51	15	39	—	—	—
Toxaphene 20	86	92	85	100	53	100	100	—	—
Sulfur 100	—	—	—	—	—	—	—	—	67[2]

[1] Rate of application 15 pounds per acre.
[2] Rate of application 25 pounds per acre.

Treatments containing 0.5 pound dieldrin also gave a higher mortality than 2 pounds of toxaphene when both were left unwashed.

Laboratory tests were also conducted to determine the effectiveness of dieldrin against boll weevils developing inside of squares. Field-collected infested squares were treated with various dust concentrations, the squares were caged, and the weevil emergence determined. It was found that dieldrin killed many of the developing forms. A dust containing 1.25 per cent of dieldrin gave a kill approximately equal to that given by dusts containing 5 or 10 per cent of chlordane which has been reported (Rainwater 1947) to be effective against weevils developing inside of squares. The 1.25 per cent dieldrin dust was somewhat more effective than a 2.5 per cent chlordane dust, and was much more effective than one containing 20 per cent of toxaphene or than sufficient benzene hexachloride to give 3 per cent of the gamma isomer.

BOLLWORM, *Heliothis armigera* (Hbn.).—A dust containing 20 per cent of toxaphene was slightly more effective against third instars of the bollworm than one containing 5 per cent of dieldrin, although the difference was not statistically significant. At concentrations of 2.5 per cent or less, dieldrin was significantly less effective than a 20 per cent toxaphene dust.

Tests with simulated rainfall were also conducted against the bollworm. In either spray or dust formulations 0.5 pound of dieldrin gave greater kill after being sprayed than did 2 pounds of toxaphene. A 0.25-pound dosage of dieldrin was somewhat less effective than toxaphene in these tests. Each of two proprietary stickers applied at the rate of 0.5 pound per acre increased the effectiveness of the 0.25-pound dosage. A liquid latex composition was used in the spray formulation, and a colloidal dust sticker in the dust. In neither instance, however, was the kill equal to that obtained with a 0.5-pound dosage of dieldrin or a 2-pound dosage of toxaphene.

TOBACCO BUDWORM, *Heliothis virescens* (F.).—A 5 per cent dieldrin dust gave slightly better results than did a 20 per cent toxaphene dust against third instars of the tobacco budworm. Lower concentrations were not tested against this species.

COTTON LEAFWORM, *Alabama argillacea* (Hbn.)—A 5 per cent dieldrin dust was also effective against fourth instars of the cotton leafworm. However, lower concentrations did not compare favorably with 20 per cent toxaphene dust against this species.

SALT-MARSH CATERPILLAR, *Estigmene acrea* (Drury).—Dieldrin dust at a concentration of 5 per cent killed 98 per cent of third instars of the saltmarsh caterpillar. Lower concentrations were much less effective than a dust containing 20 per cent of toxaphene.

VARIEGATED CUTWORM, *Peridroma margaritosa* (Haw.)—A dust containing 2.5 per cent of dieldrin killed all the third instars of this cutworm in the test. Lower concentrations were not tested.

GARDEN WEBWORM, *Loxostege similalis* (Guen.)—A 5 per cent dieldrin dust did not compare favorably with a 20 per cent toxaphene dust against fourth instars of the garden webworm. Higher concentrations were not tested.

COTTON APHID, *Aphis gossypii* Glover.—Concentrations of dieldrin ranging up to 40 per cent failed to kill more than 29 per cent of the aphids. For this reason there appears to be an excellent possibility for aphid build-up when dieldrin is used in field applications.

SPIDER MITE, *Septanychus* sp.—A spider mite was not killed by a 2.5 per cent concentration of dieldrin. The possibility of spider mite build-up after field application of this material therefore should not be overlooked. Sulfur, used for comparison in this test, gave a 67 per cent mortality.

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Late-Season Control of Boll Weevil and Bollworm with Dusts and Sprays

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Late-season damage by the boll weevil, *Anthonomus grandis* Boh., and the bollworm, *Heliothis armigera* (Hbn.), in the central Texas area during 1949 afforded an opportunity to conduct tests with various dusts and sprays for control of these insects. Although both insects were a factor in each of the experiments, the boll weevil was of primary importance in five experiments, the bollworm in one, and both of about equal importance in three. In addition to materials recommended for cotton insect control during 1949 in the various states, two comparatively new compounds, aldrin and dieldrin, were used in some of the experiments. Materials were applied as dusts in all experiments reported herein except three, in which sprays were used.

The plots in experiment 1 were 16 rows wide, 0.2 acre in size, and were arranged in randomized blocks with 4 replications of each treatment. The others were large-scale experiments in which the plots ranged from 1 to 60 acres in size. The small plots were dusted with rotary hand dust guns and the large plots with power-take-off, 6- or 8-row tractor attachment dusting machines. Emulsions were applied with 6- or 8-row tractor attachment, low-pressure and low-volume spray machines —7.5 gallons per acre at a pressure of 60 pounds per square inch. Three nozzles per row were used to apply the spray. The procedure and method of recording data were the same as those reported by Ewing & Parencia (1943). Fleahoppers did not become sufficiently numerous to cause appreciable damage in any of the experiments. The treatments and experimental data are shown in table 1.

Toxaphene dusts in the large-scale experiments were standard commercial products and were furnished by the cooperating growers. Other dust materials and emulsion concentrates were obtained from various manufacturers and their reported contents are listed in table 1.

Boll Weevil Experiments.—In experiment 1 the boll weevil infestation records show that: (1) each dust treatment with the exception of the 7.5 per cent chlordane-5 per cent DDT mixture reduced the boll weevil infestation significantly below that of the check; (2) the toxaphene and the dieldrin treatments gave significantly better boll weevil control than the two chlordane mixtures; and (3) the dieldrin treatment gave significantly better weevil control than the aldrin-DDT mixture. Each insecticide reduced the bollworm boll injury significantly below that of the check, and all dusted plots produced significantly more seed cotton per acre than the untreated check. Dieldrin-treated plots produced a significantly higher yield than any treated by the other insecticides. The lowest gains in yield were obtained with the two chlordane-DDT mixtures.

In experiment 2 the greatest reduction in weevil infestation occurred in the toxaphene treatment. Although there was no damage from the cotton aphid, *Aphis gossypii* (Glov.), the aldrin-DDT mixture caused a slight aphid build-up with a seasonal average of 1.6 aphids per square inch. The increases in yield were highest from the toxaphene and the aldrin-DDT treated plots.

In experiment 3 the best boll weevil control was obtained from toxaphene and from the mixture of benzene hexachloride, DDT, and sulfur. The aphid infestation averaged 3 per square inch in the plots treated with the 7.5 per cent chlordane-5 per cent DDT mixture, 6 in those receiving the 10 per cent chlordane-5 per cent DDT mixture, and 0.01 in those treated with toxaphene and the benzene hexachloride-DDT mixture.

Experiment 4 was located in a field of cotton planted June 22 after the harvest of a crop of sweet corn. The field was subjected to a heavy migratory weevil population, as most of the early-planted cotton in the area was mature by mid-August. The weevil infestation averaged 35 per cent in the calcium arsenate-

[1] These studies were made in cooperation with the Texas Agricultural Experiment Station.

[2] The authors acknowledge the valuable assistance given by H. S. Johnson, Jr., and C. B. Cowan, Jr., in making many of the routine field-plot records.

[3] Presented at the meeting of the Texas Entomological Society, Houston, Tex., January 19 and 20, 1950.

Table 1.—Effectiveness of several organic insecticides against boll weevils and bollworms.

Treatment	Dust or Spray, per Acre-Application	Boll Weevil Punctured Squares	Bollworm Infestation		Aphids per Square Inch	Yield of Seed Cotton per Acre	
			Injured Squares	Injured Bolls		Total	Gain Over Check
	Pounds	*Per Cent*	*Per Cent*	*Per Cent*	*Number*	*Pounds*	*Pounds*
Boll Weevil Experiments							
Experiment 1 (5 applications Aug. 3, 8, 12, 17,[1] 18)							
Toxaphene 20%+sulfur 40%	17	39	3	2	0.003	1248	405
Dieldrin 2.5%	17	32	3	3	0.016	1485	642
Aldrin 2.5%+DDT 5%+sulfur 40%	17	48	2	2	0.02	1235	392
Chlordane 7.5%+DDT 5%+sulfur 40%	16	58	2	2	0.017	1118	275
Chlordane 10%+DDT 5%+sulfur 40%	17	56	2	3	0.006	1130	287
Untreated check	—	68	4	7	0.006	843	—
Differences required for significance, 5% level	—	11	1	3	0.006	217	—
Experiment 2 (7 applications Aug. 9,[1] 11, 16, 20, 25, 31, Sept. 5)							
Toxaphene 20%+sulfur 40%	14	39	2	1	0.01	1511	847
Aldrin 2.5%+DDT 5%+sulfur 40%	14	50	2	2	1.6	1476	812
Chlordane 7.5%+DDT 5%+sulfur 40%	14	59	1	1	0.07	1305	641
Chlordane 10%+DDT 5%+sulfur 40%	14	64	2	2	0.02	1437	773
Untreated check	—	85	2	4	0.01	664	—
Experiment 3 (4 applications July 29, Aug. 3, 8, 11)							
Toxaphene 20%+sulfur 40%	17	39	2	1	0.01	1589	533
Benzene hexachloride, gamma 2%+DDT 10%+sulfur 40%	16	37	1	1	0.01	1808	752
Chlordane 7.5%+DDT 5%+sulfur 40%	17	48	1	1	3.0	1631	575
Chlordane 10%+DDT 5%+sulfur 40%	17	60	1	2	6.0	1513	457
Untreated check	—	83	1	1	0.2	1056	—
Experiment 4 (8 applications Aug. 16, 20, 25, 30, Sept. 6, 15,[1] 22, 27							
Toxaphene 20%+sulfur 40%	12	44	1	—	3.0	1605	—
Benzene hexachloride, gamma 1%+calcium arsenate	12	35	2	—	4.0	1495	—
Outside check	—	—	—	—	—	0	—
Experiment 5 (8 applications Aug. 5, 9, 15, 19, 24, 29, Sept. 2, 8)							
	Gallons						
DDT 0.69 lb., aldrin 0.34 lb.	7.5	48	1	1	0.3	1084	795
DDT 0.69 lb., chlordane 1.38 lb.	7.5	66	1	1	1.1	809	520
DDT 0.69 lb., toxaphene 1.38 lb.	7.5	59	0.3	0.3	0.3	873	584
Untreated check	—	80	1	1	0.02	289	—
Bollworm Experiment							
Experiment 6 (4 applications July 13, 19, 28, and Aug. 3)							
Toxaphene 2 lb., DDT 1 lb.	7.5	7	2	4	—	1340	264
Toxaphene 1 lb., DDT 0.5 lb.	7.5	10	3	5	—	1292	216
Toxaphene 2 lb.	7.5	9	3	5	—	1233	157
Toxaphene 1 lb.	7.5	12	5	8	—	1121	45
DDT 1 lb.	7.5	8	2	2	—	1350	274
Untreated check	—	14	4	6	—	1076	—
Difference required for significance, 5% level	—	—	1	2	—	97	—
Boll Weevil-Bollworm Experiments							
Experiment 7 (4 applications July 22, 27, Aug. 3, 9)							
Toxaphene 20%+sulfur 40%	15	35	2	4	—	1156	72
	Gallons						
Toxaphene 1.5 lb.+DDT 0.75 lb.	7.5	40	2	2	—	1425	341
Untreated check	—	52	3	4	—	1084	—
Experiment 8 (4 applications July 28, Aug. 2, 8, 13)							
	Pounds						
Benzene hexachloride, gamma 2%+DDT 10%+sulfur 40%	14	29	1	1	—	1105	455
Untreated check	—	49	2	4	—	650	—
Experiment 9 (4 applications July 30, Aug. 4, 9, 16)							
Toxaphene 20%+sulfur 40%	18	33	1	3	—	1172	522
Benzene hexachloride, gamma 2%+DDT 10%+sulfur 40%	18	27	1	4	—	1245	595
Untreated check	—	49	2	4	—	650	—

[1] Washed off by rain within 24 hours.

gamma benzene hexachloride mixture and 44 per cent in the toxaphene treatment, whereas the aphid infestation averaged 4 and 3 per square inch, respectively. Although no untreated check was included in the experiment, a field of cotton planted on the same date on a neighboring farm in the same creek bottom, because of weevils, did not produce enough cotton to be picked by the grower. Plots dusted with the calcium arsenate-benzene hexachloride mixture produced 1495 pounds of seed cotton per acre and those dusted with toxaphene 1605 pounds.

Experiment 5 was located in a field of June-planted cotton. The best weevil control was obtained from the aldrin-DDT spray with a seasonal average of 48

per cent of punctured squares compared with 59 per cent in the toxaphene-DDT spray, 66 per cent in the chlordane-DDT spray and 80 per cent in the untreated check. The gain in yield of seed cotton per acre in the treated plots ranged from 520 to 795 pounds compared with only 289 pounds produced in the check plot.

BOLLWORM EXPERIMENT.—The plots in experiment 6 were 32 rows wide, and were arranged in randomized blocks with 4 replications of each treatment. Two replicates of each treatment were located in each of two fields. Two spray applications were made in the experiments early in the season (June 3 and 9) for thrip control at one-half the dosage listed in the table for bollworm control. The thrip infestation was light and the 2 early treatments had little, if any, effect upon the final results of the experiment. Four spray applications were made for bollworm control between July 13 and August 3. Each treatment except toxaphene applied at the rate of 1 pound per acre reduced the bollworm injury in the squares and in the bolls significantly below that of the untreated check. The emulsion containing DDT alone reduced the boll injury significantly below that of the other emulsions except the toxaphene-DDT (2 to 1) emulsion. Plots treated with each spray except the 1 pound of toxaphene produced significantly more seed cotton per acre than the untreated check. All the treated plots produced yields that were significantly higher than that produced by 1 pound of toxaphene. The two dosages of toxaphene plus DDT and DDT alone each produced significantly higher yields than toxaphene at 2 pounds per acre.

COMBINATION BOLL WEEVIL AND BOLLWORM EXPERIMENTS.—In experiment 7 a toxaphene dust was compared with a toxaphene-DDT spray. The dust gave better control of boll weevils and the spray gave slightly better control of bollworms.

In experiment 8 a dust containing the gamma isomer of benzene hexachloride, DDT, and sulfur gave good control of boll weevils and bollworms. The gain of seed cotton per acre was 455 pounds more than that of the untreated check.

In experiment 9 somewhat better boll weevil control was obtained from a dust mixture containing the gamma isomer of benzene hexachloride, DDT and sulfur than from the toxaphene dust, with a slight difference in bollworm control in favor of toxaphene. Gains in yield—pounds of seed cotton per acre—over that of the untreated check were 595 and 522 pounds, respectively.

SUMMARY.—Insecticides applied as dusts and as low-volume sprays were tested in a single small-plot and in several large-scale field experiments at Waco, Texas, during 1949 against late-season infestations of the boll weevil, *Anthonomus grandis* (Boh.), and the bollworm, *Heliothis armigera* (Hbn.). A 2.5 per cent dieldrin dust gave better results than a 20 per cent toxaphene dust in the small-plot experiment conducted for boll weevil control. A dust containing 2.5 per cent of aldrin, 5 per cent of DDT, and 40 per cent of sulfur gave results approximately equal to that of a 20 per cent toxaphene dust in two boll weevil experiments. In each of three experiments square-infestation records showed that 20 per cent toxaphene dust gave better control of the boll weevil than did 10 or 7.5 per cent chlordane mixed with 5 per cent DDT. In the small-plot experiment the yield was higher, but not significantly higher, from the toxaphene than from the chlordane-DDT mixtures. Aphid, *Aphis gossyppi* (Glov.), infestations approaching the damage point developed after the applications of chlordane-DDT dust mixtures in one of the large-scale experiments. In one experiment aldrin-DDT applied as a spray gave better boll weevil control and higher increases in yield than toxaphene-DDT or chlordane-DDT applied as sprays. Toxaphene spray at 2 pounds per acre did not give so good bollworm control in one experiment as did a spray containing 1 pound of DDT per acre. A toxaphene dust gave somewhat better boll weevil control than a toxaphene-DDT spray, but there was a slight difference in favor of the spray in bollworm control.

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Comparison of Early-Season, Late-Season, and a Combination of Early-Season Plus Late-Season Insecticide Applications for Cotton Insect Control

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Early-season insects damage cotton in the central Texas area every year. These insects include thrips (several species), cotton fleahoppers *Psallus seriatus* (Reut.) and overwintered boll weevils, *Anthonomus grandis* Boh. They often delay the fruiting and maturity of the cotton by two or three weeks (Ewing & Parencia 1949), and the delay in maturity leaves the cotton exposed for a longer period to attack from late-season insects such as boll weevils and bollworms, *Heliothis armigera* (Hbn.).

An infestation of thrips and overwintered boll weevils in many fields in the Waco area during 1949 afforded an opportunity to test several of the organic insecticides, alone or in combinations, applied as concentrated sprays against these insects, and to compare results secured from early-season and late-season insecticide applications for cotton-insect control. In some tests dusts and sprays were compared. Six experiments were conducted on various farms in the Brazos River Bottom and in the Prairie Blacklands near Waco. While it was known that best results from early-season insecticide applications, especially for boll weevil control, were realized if large areas were treated (Ewing *et al.* 1947, Ewing & Parencia 1949), it was felt that much information could be obtained even on a small-scale or field-plot basis.

Materials and Procedure.—One to three insecticidal treatments were compared with an untreated check in each field. The plots ranged from 2 to 16 acres in size. The experiments were designed so that, when insect infestations warranted control later in the season, the plots could be subdivided and a portion of each plot treated while the other portion was left untreated. Need for late-season treatment was based on infestation counts. Thus some of the plots or subplots received no treatment throughout the season, some received early treatment only, some received both early and late treatment, and some received late treatment only.

Three dusts were used in the experiments, one containing 20 per cent of toxaphene plus an inert filler, another containing 20 per cent of toxaphene plus 40 per cent of sulfur, and a mixture containing 10 per cent of DDT, 40 per cent of sulfur, and enough benzene hexachloride to give 2 per cent of the gamma isomer in the finished dust. The emulsion concentrates consisted of toxaphene 6 pounds per gallon, toxaphene 4 pounds plus DDT 2 pounds per gallon, and chlordane 4 pounds plus DDT 2 pounds per gallon.

The dusts were applied by 6- or 8-row ground machines. The early-season applications were made at an approximate rate of 10 rounds per acre. Late-season applications were at about 15 pounds pers acre.

The emulsions were applied by 6- or 8-row tractor attachment machines. The total amount of spray used in the early treatments, 2.5 gallons per acre, was applied at a pressure of 60 pounds per square inch with one nozzle per row. From 1 to 2 pints of the emulsion concentrate was used per acre. Late-season applications were made at a rate of 7.5 gallons of liquid per acre at a pressure of 60 pounds with three nozzles per row. The amount of emulsion concentrate used ranged from 3 pints to 2 quarts per acre.

The early-season applications were made between May 12 and June 8, with the exception of the last early-season application in one experiment, which was made on June 20. The late applications were made between July 8 and 29. Dusts were applied early in the morning, when the best conditions for dusting usually prevail in this area. The emulsions were applied during daylight hours. Wind velocities ranged from very low to as high as 20 miles per hour during the time that spray applications were made.

Results.—The results of the six experiments are shown in table 1. In the

[1] In cooperation with the Texas Agricultural Experiment Station.

[2] H. S. Johnson, Jr., and C. B. Cowan, Jr., assisted in making many of the routine field-plot records.

first group of four experiments good control of early-season insects, such as thrips and overwintered boll weevils, was secured from the early-season treatments. Excellent control of the cotton fleahopper was also obtained (not shown in table). The effective early-season insect control is reflected in the bloom records (4 records made over a period of 15 days prior to July 9), which show an average of slightly more than twice as many blooms in the plots receiving early treatment as in the plots receiving no treatment. In each of these experiments the results of thrips control could readily be seen in the appearance of the plants a few days after treatments were started. The setting of the crop began two to three weeks earlier in the early treated plots.

While the seasonal boll weevil infestation was comparatively low, late in the season the infestations in all the untreated plots reached the damaging level of 25 per cent of squares punctured with a maximum of 85 per cent in one experiment. In the plots receiving early treatments only the weevil infestation reached the 25 per cent level in three of these four experiments.

In one of the experiments the level of boll weevil infestation was 78 per cent of squares punctured on July 22 in an early treated plot that adjoined an untreated plot. On the same date the infestation level was only 20 per cent of squares punctured in another early treated plot located at some distance from the untreated plot. This experiment illustrates the need for treating at least entire fields with early-season applications for boll weevil control.

A light to medium bollworm infestation had developed on all the plots at the time the late treatments in the subplots were started. Although damage was comparatively light, it was about twice as great in the plots receiving no late treatment as in the plots receiving late treatment.

The plots receiving an average of 3.5 early-season applications produced 317 pounds more seed cotton per acre than the untreated checks, plots receiving an average of five late-season applications produced a gain of 379 pounds, and plots receiving an average of 7.3 early plus late applications produced a gain of 460 pounds. The results of these experiments indicate that early-season insecticide applications may be more economical than late-season applications in the Central Texas area, even though they are made on a field or small-farm basis. Early-season insect control becomes even more important when the value of early production and early harvesting of the crop is considered. Late-season applications, especially on highly productive land, are likely to be needed after the early-season applications on areas smaller than entire farms or communities.

In the second group of two experiments results were similar to those of the first group. The plots were not subdivided for late-season control, because boll weevil and bollworm infestations did not become severe enough in the treated plots late in the season to warrant control measures. The plots receiving early treatment produced an average of 300 pounds of seed cotton per acre more than the check plots.

In two of the experiments toxaphene was applied as dust and as spray. Control of thrips, overwintered weevils, and other early-season insects was approximately the same from the two methods. The spray caused a gain in yield of 315 pounds and the dust a gain of 318 pounds of seed

Table 1.—Summary of records made in experiments to compare early-season, early- plus late-season, and late-season insecticide treatments for cotton-insect control.

Time of Treatment	Number of Applications	Thrips per Plant	Boll Weevils per Acre	Blooms per Acre	Per Cent of Weevil-Punctured Squares		Per Cent of Bollworm-Injured Squares	Per Cent of Bollworm-Injured Bolls	Yield of Seed Cotton per Acre	
					Entire Season	Late-Season			Total	Gain Over Check
Group 1 (4 experiments, four replicates of each experiment)										
Early	3.5	2	10	3725	15	34	6	7	1353	317
Early and Late	7.3	2	10	3725	9	15	3	3	1496	460
Late	5	8	47	1821	18	29	2	3	1415	379
Check (No Treatment)	—	8	47	1821	20	39	5	6	1036	
Group 2 (2 experiments, two replicates of each experiment)										
Early	3	1	5	3789	—	21	1	2	1080	300
Check (No Treatment)	—	8	40	1466	—	46	1	3	780	

cotton per acre over the untreated check.

SUMMARY.—Field-plot experiments have been conducted in the Waco area during 1949 to compare early-season, late-season, and early- plus late-season insecticide applications for cotton insect control.

The cotton which received early-season treatment set and matured its crop two to three weeks earlier than cotton which received no early treatment. Control of thrips (several species), overwintered weevils, *Anthonomus grandis* Boh., and other early-season insects was approximately the same when a dust and a spray were compared. Plots receiving an average of 3.5 early-season applications produced a gain of 317 pounds of seed cotton per acre over plots receiving no treatment, plots receiving an average of 5 late-season applications produced a gain of 379 pounds, and plots receiving an average of 7.3 early plus late applications produced a gain of 460 pounds.

The results of these experiments indicate that early-season insecticide applications may be more economical than late-season applications in the Central Texas area even though entire farms or communities are not treated.

Late-season applications, especially on highly productive land, are likely to be needed after early-season applications on areas smaller than entire farms or communities.

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Comparative Effectiveness of Aldrin Applied at Different Times of the Day for Boll Weevil Control[1]

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Aldrin was used experimentally against cotton insects in 1948, but no attempt was made to determine its performance under the various climatic conditions. In 1949 a large-scale experiment was conducted at Stoneville, Miss., to obtain information on the climatic factors normally encountered under field conditions when aldrin was used as a spray.

Temperature, humidity, wind movement, convection currents, and light intensity have been shown to influence the toxicity of all the organic insecticides recommended for cotton-insect control. Apparently the toxicity of the insecticides that have a high fumigating action is more adversely affected by these factors than that of insecticides having a low vapor pressure. Gaines & Dean (1949) found that the toxicity of toxaphene was reduced appreciably by high temperatures, but not so much as that of a mixture containing 3 per cent of gamma benzene hexachloride and 5 per cent of DDT. This mixture was so greatly reduced in toxicity by high temperatures that dosages as high as 30 pounds per acre were necessary to obtain a high mortality of the boll weevil. Dustan *et al.* (1947) reported the fumigating action of benzene hexachloride to be greatly reduced even when exposed to air currents of less than 3 miles per hour. Smith *et al.* (1948) concluded that as an aphicide low dosages of benzene hexachloride were approximately twice as effective when applied in the evening as when applied in the morning. They suggested that humidity, dew, wind, temperature, and convection currents in the field also reduced the effectiveness of applications made early in the morning for aphid control.

Most of the insecticides used for the control of cotton insects in the last 30 years have been applied in dust form. Because of unfavorable climatic conditions during most of the day, it has often been

[1] Certain phases were carried on under the Research and Marketing Act of 1946, in cooperation with the Delta Branch of the Mississippi Agricultural Experiment Station.

[2] R. V. McGahey and M. D. Dick assisted with these investigations.

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necessary to confine the applications of poison to a few hours in the early morning, late afternoon, or evening. Brooks (1947) working with airplane dustings to alfalfa found that a total off-tract waste of 50 to 60 per cent of the insecticide resulted from drift caused by air currents.

Recent work by Calhoun (1949), Calhoun & Smith (unpublished), Stevenson & Kaufman (1948), Ewing & Parencia (unpublished), and Parencia *et al.* (1946) indicate that some of the organic insecticides are very effective against several cotton insects when applied as low-volume sprays and that sprays can be applied throughout most of the daylight hours even in relatively strong winds. These findings suggested that more information was needed on the influence of the various climatic factors, which change almost hourly throughout the day, on the effectiveness of insecticides applied as sprays for the control of the boll weevil, *Anthonomus grandis* Boh. In carrying out these studies incidental information was also obtained on the control of the cotton aphid, *Aphis gossypii* Glov., and the bollworm, *Heliothis armigera* (Hbn.).

METHODS AND MATERIALS.—Aldrin was used in an emulsion at the rate of 0.2 pound in 1 gallon of spray per acre. Information obtained from the manufacturer suggested that aldrin is slightly less volatile than benzene hexachloride and has a residual action almost equal to that of chlordane. An airplane,[1] equipped with a gravity-flow rotating-disk-type spray unit, was used to apply the insecticide at 6 and 10 A.M., and at 2 and 6 P.M. at intervals of 5 to 7 days from July 26 to September 3. Nine applications were made during this period. The plane was usually flown from 5 to 8 feet above the tops of the cotton plants. Spray coverage was obtained over a swath width of approximately 33 feet. By increasing the number of disks on the spray unit from two to eight, the mass average droplet size could be reduced from 500 microns to almost that of an aerosol. Mass average droplet size of about 200 microns was used throughout the test. DDT in an emulsifiable form was added to aldrin at the rate of 0.5 pound per acre in each of the last three applications for control of the bollworm. The total amount of spray applied per acre was not changed by the addition of the DDT. With the exception of the 6-A.M. treatment on August 19, which was washed off by 0.25-inch rain within 2 hours after application, all treatments were effective. The 6-A.M. treatment was repeated the following morning. On 2 days late in August the air became so rough in midmorning and midafternoon that some minor changes were necessary in the time of application.

The test was located in a 160-acre field of very uniform cotton planted on May 16 and 17. The field was divided into 4 randomized blocks of 40 acres each. Each treatment was applied to one 10-acre plot in each block. Flagmen were used to direct the pilot and to insure proper distances between swaths. A single outside check, 1 acre in size but comparable to the treated plots in soil fertility, plant growth, and early weevil infestation, was used to determine the effectiveness of the insecticide. In analyzing the data the check was not included.

Squares were examined at weekly intervals to determine the per cent of boll weevil infestation, the number of live weevils per 100 squares, and the per cent of squares damaged by the bollworm. A single observation was made in which 1 square inch of the under surface of 100 leaves was observed in each plot to determine the population of aphids. Observations were made on temperature, relative humidity, and wind movement while the insecticides were being applied and on rainfall throughout the test period. Records were also obtained on the total wind movement occurring 12 hours after each application. Yield records were obtained by picking two hundredth-acre areas in each 10-acre plot and in the check.

RESULTS.—There was a significant difference at the 5 per cent level between the boll weevil infestation in the plots treated at 6 P.M. and in those treated at 2 P.M. and 6 A.M. (Table 1). The infestation after the 6-P.M. treatment was not quite significantly better than that after the 10-A.M. treatment, but after August 2 every infestation record was lower in plots treated at 6 P.M. The average infestation in the check was 50 per cent higher than the highest average infestation in the treated plots, and gave a measure of the degree of control obtained with all treatments.

[1] This plane Piper PA-11 was made available by the Delta Branch of the Mississippi Agricultural Experiment Station through the cooperation of the Piper Aircraft Corporation.

Table 1.—Effectiveness of aldrin for aphid, bollworm, and boll weevil control when applied at different times of the day by airplane.

Time of Treatment	Aphids per Square Inch of Leaf	Bollworm Infestation in Squares	Boll Weevils per 100 Squares	Boll Weevil Infestation in Squares	Yield of Seed Cotton per Acre
	Number	*Per cent*	*Number*	*Per cent*	*Pounds*
Check	2.4	1.10	10.0	81	1399
6 A.M.	1.1	.01	1.1	31	2129
10 A.M.	2.8	.03	1.8	26	2055
2 P.M.	1.4	.02	1.0	31	2171
6 P.M.	.5	.03	.9	18	2122
Differences required for significance:					
At 5% level				9.4	338
At 1% level				12.9	

The total wind movement over the treated field the first 12 hours after each application of the insecticide is shown in figure 2. The fact that there was only one-third as much wind movement in the 12 hours after the 6-P.M. treatment as after the 6-A.M. treatment may have affected the boll weevil control obtained. Since other tests have shown that 59 per cent of the adult boll weevils killed by aldrin died within 8 hours after the application, this material may have very strong fumigating action and thus be dissipated rapidly by strong air movement. The very heavy dew which occurred throughout most of the season may also have had some influence on the control obtained from the 6-A.M. treatment. The further dilution of the insecticide by the dew may have contributed to the decreased control recorded.

The pattern of control for the 10-A.M. and 2-P.M. treatments changed after August 22 (figure 1). Cold fronts moved through this area on August 21 and 28, causing strong convection currents from midmorning until midafternoon for several days. It is possible that these currents prevented small droplets from being deposited on the cotton plants and dissipated the insecticide vapor more rapidly immediately after application. Flying conditions during this period were very unfavorable.

Yield records showed no significant differences between treatments. This can be readily understood when it is considered that the cotton plant normally sheds 50 per cent or more of the fruit forms produced, and the highest average infestation in any of the treated plots was far below this 50 per cent point. A comparison of the treated plots with the check plots showed that the former produced from 656 to 772 pounds more seed cotton than the latter. In this comparison the earlier cited difference in infestation reflected the difference in yield.

The cotton aphid was present in all

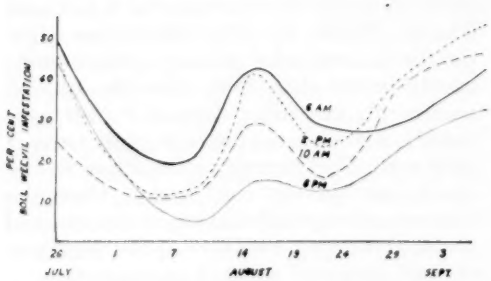

FIG. 1.—Boll weevil infestation after treatments with aldrin applied at different hours of the day.

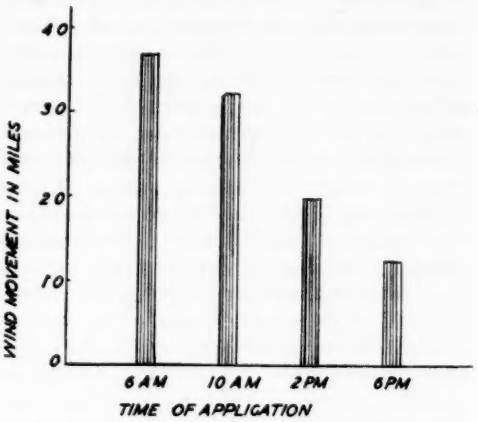

FIG. 2.—Total wind movement across field during first 12 hours after application of insecticide.

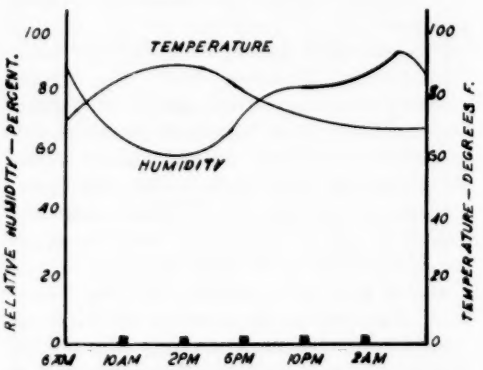

Fig. 3.—Average daily fluctuation of temperature and relative humidity recorded on dates poison applications were made from July 26 to September 3.

plots from the beginning of the experiment but did not develop in sufficient numbers to cause economic damage. Fewer aphids were present after the 6-P.M. treatment than after the others but the difference between treatments was not significant.

The bollworm infestation remained low throughout the test. DDT was included in the last three poison applications as a precautionary measure, because numerous bollworm eggs and young larvae were observed in some plots on August 23, and aldrin had not been found to be very effective against the bollworm in previous tests.

No significant difference was observed between treated plots in the average number of live boll weevils found in each 100 squares. In contrast there were eleven times as many live weevils in the check plot as in the plots treated at 6 P.M.

Rainfall was very light throughout the test, totaling only 1.85 inches.

The average temperature and average relative humidity are shown in figure 3.

CONCLUSIONS.—Aldrin at the rate of 0.2 pound of toxicant in 1 gallon of spray per acre was more effective for boll weevil control when applied at 6 P.M. than at any other time of the day. It was apparent in this test that several factors adversely influenced the weevil control obtained at 6 and 10 A.M., and at 2 P.M. Dew, wind movement, temperature, and convection currents were all possible contributing factors, but it cannot be said that any one factor was responsible for the reduction in control. The fact that there was no significant difference in yield indicates that aldrin, in the amount used was sufficiently toxic to the boll weevil in all treatments to hold the infestation below the level at which a reduction in yield could occur, despite the adverse influence of certain climatic factors.

SUMMARY.—A large-scale field test was conducted near Stoneville, Miss., in which aldrin was applied nine times from July 26 to September 3 as an emulsion spray by airplane for control of the boll weevil, *Anthonomus grandis* Boh. Applications were made at 6 and 10 A.M. and 2 and 6 P.M. to determine the influence of climatic factors occurring at different times of the day on toxicity of the insecticide.

Boll weevil control was significantly better after the 6-P.M. treatment than after the 6-A.M. or 2-P.M. treatments, but not quite significantly better than after the 10-A.M. treatment. There was no significant difference in yield between the treated plots, because all held the infestation sufficiently low to prevent a reduction in yield. The treated plots yielded from 656 to 772 pounds more seed cotton per acre than the untreated check plot.

Wind movement, temperature, convection currents, and dew were suggested as factors contributing to the differences observed in boll weevil control.

The cotton aphid, *Aphis gossypii* Glov., did not develop in sufficient numbers to be of economic importance. DDT was used to prevent a threatened infestation of bollworm, *Heliothis armigera* Hbn.

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Effect of Climatic Factors on the Toxicity of Certain Insecticides[1]

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Results of toxicity tests conducted at this Station to determine the effects of temperature and humidity on several insecticides applied as dusts have been reported by Gaines & Dean (1949). These investigations have been continued using insecticides applied as sprays and dusts. Little information has been reported regarding the residual toxicity of organic insecticides applied as emulsions. The work herein reported was conducted during 1949 to determine the effect of temperature, humidity, dew, wind and sunshine on the toxicity of toxaphene dust and spray, toxaphene-DDT spray and gamma benzene hexachloride dust and spray.

Methods.—The dusting apparatus, chamber, etc., used to apply insecticidal dusts were described in the paper referred to above.

A spraying apparatus was developed with which sprays could be applied at 60-pound pressure with each nozzle delivering about 2.5 gallons of spray per acre when the machine was operated at approximately 5 miles per hour. An old automobile differential gear was utilized to make a motor-driven turntable. One end of the axle was cut off and inserted in a metal cup resting on the base. The other wheel was replaced with a wooden disc over which a steel frame was made to accommodate 4 pot plants in a circle about 9 feet apart. A motor attached to the drive shaft operating at a certain speed furnished the power to drive the turntable. By arranging the pots at different distances from the center of the turntable various speeds could be obtained. Spraying of the potted plants while they were in motion was accomplished by operating a spray gun-air-tank assembly which was mounted on a movable stand. The cone nozzles could be adjusted to give adequate coverage of the plant. The spray gun consisted of a small copper tank with 2 nozzles and an air supply from a tank equipped with a regulator valve, cut off, etc. The spray was released while the plants were being rotated at a constant speed. This method compared favorably to a tractor spray machine operated in the field.

The boll weevils, *Anthonomus grandis* Boh., used in all tests were field-collected while the leafworms were laboratory-reared. Survival records were made daily for a period of 5 days after each treatment. The same number of replicates of each insecticide was included in each experiment, making the tests comparable from the standpoint of population as well as that of the condition under which the test was conducted. Four replicate cages of each dose were included and 4 cages of untreated insects served as checks. A minimum of 5 dosages, which included 20 cages or 300 individuals, were used, to establish each dosage-mortality curve. Abbott's formula was used to calculate the percentage mortality. The dosages were calculated to the acre basis and changed to logarithms and the percentage mortalities were changed to probits for calculating the dosage-mortality curves. The regression lines were calculated according to Bliss' (1938) method.

Hygrothermographs were used to record the temperature and relative humidity during the duration of each experiment.

Simulated dews were applied by allowing a fine mist to settle on the plants at about the same time dew would normally form on plants in the field.

The sprays were made by diluting miscible oil concentrates with water. The 3 to 5 sprays and dusts contained 3 per cent gamma benzene hexachloride-5 per cent DDT. The toxaphene and DDT were mixed in a 2 to 1 ratio. The total active ingredients are shown for the mixtures.

Results.—Experiment 1, figure 1, was conducted in the laboratory. The average daily temperatures during the test period ranged from 76.2 to 91.3° F. and the average daily relative humidities ranged from 39.5 to 82.4 per cent. The median lethal

[1] Technical Contribution No. 1316, Texas Agricultural Experiment Station in cooperation with the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.

Table 1.—Median lethal doses in pounds active ingredients for each insecticide tested against the boll weevil under conditions indicated at College Station.

Treatment	Laboratory[1]		Greenhouse[2]	
	MLD	Slope of Line	MLD	Slope of Line
Toxaphene				
Dust	.15	2.09	.08	3.22
Spray	.16	6.33	.07	3.04
Toxaphene-DDT (2–1)				
Spray	.20	.70	.08	4.60
Benzene hexachloride-DDT (3–5)				
Dust	.20	1.82	.10	3.55
Spray	.09	3.35	.07	2.67

[1] Average daily temperatures: Min. 76.2°, Max. 91.3° F.
Average daily relative humidity: Min. 35.5, Max. 82.4%
[2] Average daily temperatures: Min. 75.0°, Max. 103.5° F.
Average daily relative humidity: Min. 31.0, Max. 86.5%

dose (MLD) and regression coefficient are given for each insecticide in table 1. The gamma benzene hexachloride-DDT spray was the most effective insecticide used as indicated by the low MLD. The toxaphene spray was as effective as the dust at the 50 per cent mortality level while at the 95 per cent level the spray was more effective. The toxaphene-DDT (2–1) was not as effective as the toxaphene

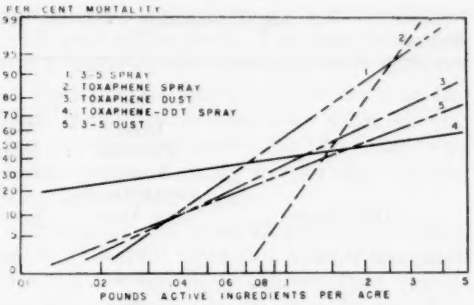

FIG. 1.—Dosage-mortality curves for the toxicity of gamma benzene hexachloride-DDT (3–5) spray and dust, toxaphene spray and dust and toxaphene-DDT (2–1) spray to field-collected boll weevils in experiment 1 conducted in the laboratory.

alone when applied at the same rate. The DDT in the mixture reduced the weevil mortality under the conditions of this experiment.

Experiment 2, figure 2, was conducted in the greenhouse where the cages were shaded and not exposed to the direct rays of the sun. The average daily temperatures during the test period ranged from 75 to 103.5° F. and the average daily relative humidities ranged from 31.0 to 86.5 per cent. The median lethal dose

Table 2.—Boll weevil mortality following treatments as indicated in the laboratory at College Station.

Treatment	Dose Pounds Active Ingredients per Acre	Per Cent Mortality				
		Weevils Released Immediately After Treatment	Exposed to Sun 4 Hrs.[1]		No Sun	
			1 Dew[2]	2 Dews[3]	1 Dew[2]	2 Dews[3]
Toxaphene Dust	.15	51.9				
	.20	60.1	76.9	12.9	9.6	2.3
	.25	83.0				
G. Benzene hexachloride-DDT Dust (3–5)	.32	32.1	39.6	9.2	0.0	8.0
	.48	84.5				
Toxaphene Spray	.12	13.3				
	.21	86.7				
	.24	84.6	64.1	28.0	11.4	20.5
Toxaphene-DDT Spray (2–1)	.21	18.9				
	.24	60.1				
	.30	67.4	60.9	18.5	63.8	31.5
G. Benzene hexachloride-DDT Spray (3–5)	.11	50.4				
	.13	0.0	53.6	20.9	13.2	0.0
	.17	93.6				

Average temperature: Min. 75.4°, Max. 92.9° F
Average relative humidity: Min. 34.9, Max. 78.8%.
[1] Wind velocity when exposed to sun .475 miles per hour.
[2] Weevils released 24 hours after treatment.
[3] Weevils released 28 hours after treatment.

Table 3.—Boll weevil mortality following treatments as indicated in the greenhouse at College Station.

Treatment	Dose Pounds Active Ingredients per Acre	Weevils Released Immediately After Treatment	Per Cent Mortality			
			Exposed to Sun 4 Hrs.[1]		No Sun	
			1 Dew[2]	2 Dews[2]	1 Dew[2]	2 Dews[3]
Toxaphene Dust	.075	55.0				
	.125	59.4				
	.20		67.5	0.0	84.6	3.3
G. Benzene hexachloride-DDT Dust (3–5)	.10	64.0				
	.16	74.0				
	.32		75.1	0.0	80.2	0.0
Toxaphene Spray	.06					
	.24	46.2	80.3	39.0	74.0	25.6
Toxaphene-DDT Spray (2–1)	.09	76.9				
	.12	77.9				
	.24		66.3	12.4	85.4	31.0
G. Benzene hexachloride-DDT Spray (3–5)	.05	23.6				
	.11	67.0				
	.13		70.8	71.4	57.6	3.8

Average temperature: Min. 73°, Max. 100.7° F. Average relative humidity: Min. 30.4, Max. 86.4%.
[1] Wind velocity when exposed to sun .572 mile per hour. [2] Weevils released 24 hours after treatment.
[3] Weevils released 48 hours after treatment.

(MLD) and regression coefficient are given for each insecticide in table 1. The temperature under which these tests were conducted, about 12° higher than was maintained in experiment 1, seemed to increase the toxicity of all the materials used. The toxaphene-DDT spray was considerably more effective at this temperature than at the lower temperature maintained in experiment 1. There was little difference in the toxicity of toxaphene-DDT spray, toxaphene spray and dust at all levels of mortality under the conditions of this experiment. The benzene hexachloride-DDT spray was slightly more effective than the benzene hexachloride-DDT dust.

Experiment 3, table 2, was conducted in the laboratory under the conditions shown in the table. It will be noticed that the toxicity of the insecticides was not greatly affected in 24 hours as indicated by a 24-hour delay in releasing the weevils, even with a simulated dew and a 4-hour sun exposure. However, the toxicity was reduced in 48 hours by sunshine and 2 simulated dews. In general, the toxaphene and toxaphene-DDT sprays re-

Table 4.—Median lethal doses in pounds active ingredients for each insecticide tested against the leafworm in the laboratory at College Station.

Treatment	MLD	Slope of Line
Toxaphene		
Dust	.07	4.59
Spray	.01	2.79
Toxaphene-DDT (2–1)		
Spray	.01	1.74

Average daily temperature: Min. 67.4°, Max. 77.0° F.
Average daily relative humidity: Min. 46.5, Max. 64.3%.

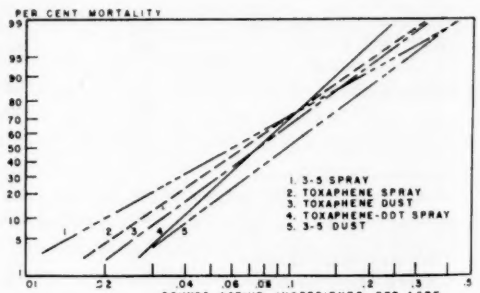

FIG. 2.—Dosage-mortality curves for the toxicity of gamma benzene hexachloride-DDT (3–5) spray and dust, toxaphene spray and dust and toxaphene-DDT (2–1) spray to field-collected boll weevils in experiment 2 conducted in the greenhouse.

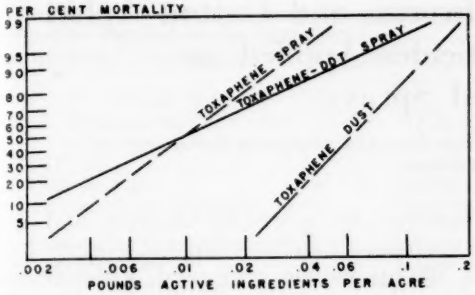

FIG. 3.—Dosage-mortality curves for the toxicity of toxaphene spray and dust and toxaphene-DDT (2–1) spray to laboratory-reared leafworms in experiment 5 conducted in the laboratory.

mained more toxic under these conditions than the toxaphene dust or benzene hexachloride-DDT dust or spray.

Experiment 4, table 3, was conducted in the greenhouse under the conditions shown in the table. Under high temperatures the toxicity of the insecticides was reduced in 24 hours by 4 hours of sunshine and a simulated dew. Again the toxaphene and toxaphene-DDT sprays remained more toxic under these conditions than toxaphene or benzene hexachloride-DDT dust.

Experiment 5, figure 3, was conducted in the laboratory to compare the toxicity of toxaphene spray, toxaphene-DDT spray and toxaphene dust against laboratory-reared leafworms, *Alabama argillaceae* (Hbn.). These tests were conducted and the dosage-mortality curves were calculated in a similar manner to those described for the boll weevil. Average daily temperatures ranged from 67.4 to 77.0° F. and the average daily relative humidities ranged from 46.5 to 64.3 per cent. The median lethal dose and regression coefficient are given for each mortality curve in table 4. The toxaphene and toxaphene-DDT sprays were considerably more effective than toxaphene dust at the 50 per cent mortality level. The low temperatures apparently decreased the toxicity of the sprays more than the dust at the high mortality levels. The slope of the dosage-mortality curve for the toxaphene dust was considerably steeper than for the toxaphene-DDT spray.

SUMMARY.—The results of cage toxicity tests with boll weevils under the conditions of average daily temperatures ranging from 76 to 91° F. are shown. The toxicity of benzene hexachloride-DDT spray was greater than either benzene hexachloride-DDT dust, toxaphene spray or dust or toxaphene-DDT spray at the 50 per cent mortality level. The toxaphene spray was equally as toxic as the benzene hexachloride-DDT at the high levels of mortality.

The results of similar tests with boll weevils under the conditions of average daily temperatures ranging from 75 to 103° F. are shown. The toxicity of all materials was increased by the higher temperatures. There was little difference in the toxicity of toxaphene-DDT spray, toxephene spray and dust at all levels of mortality. The benzene hexachloride-DDT spray was slightly more effective than the benzene hexachloride-DDT dust.

Exposing the treated plants to sunshine and simulated dews reduced the toxicity of all insecticides used. In general, the toxaphene and toxaphene-DDT sprays remained more toxic when exposed to simulated dews and sunshine than toxaphene dust or benzene hexachloride spray or dust.

In tests with leafworms under conditions of average daily temperatures ranging from 67 to 77° F., the toxaphene spray and toxaphene-DDT spray were more effective than toxaphene dust.

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Control of Boll Weevil, Bollworm, and Cotton Aphid with Organic Insecticides Applied as Concentrated Sprays[1]

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Sprays for the control of cotton insects, particularly the boll weevil, *Anthonomus grandis* Boh., the bollworm, *Heliothis armigera* (Hbn.), and the cotton aphid, *Aphis gossypii* Glov., have received but little experimental attention for many years. Most commercial control of cotton pests has been accomplished through the use of dusts. This was probably due to the general employment of arsenical poisons, which give better control of the boll weevil when applied as dusts than as sprays. The development of organic insecticides in recent years opened new avenues for the possible use of concentrated sprays in cotton insect control. Parencia *et al.* (1946) found in cage experiments that DDT sprays gave quicker kill of bollworms when applied in atomized Diesel-oil than when applied in a dust. In limited tests in Arizona, Stevenson & Kaufman (1948) reported approximately 80 per cent control of stink bugs with a concentrated spray of benzene hexachloride. This was applied by airplane at the rate of 3.5 gallons of spray per acre, and contained 4 ounces of the gamma isomer.

The use of toxaphene, DDT, chlordane, and other organic insecticides in sprays, both as suspensions and as emulsions, for the control of insects on many crops and in many parts of the world had proved to be of great value. In most instances where it has been possible to apply insecticides as sprays rather than as dusts, superior control has been obtained. Reported herein are results of preliminary tests made in 1948 at Belzoni, Rolling Fork, and Stoneville, Miss., in which concentrated sprays were applied by airplane and by ground equipment for the control of cotton insects. The dosage of toxicant was the same as has usually been employed when the same insecticide was used applied as a dust. The formulations tested are given in table 1.

Equipment.—The sprays were applied by airplanes and by ground equipment. In experiment 1 a Stearman PT 17 biplane powered by a 220-hp. engine, and in experiment 2 an N3N biplane powered by a 300-hp. engine were used. Pressure to force the spray through the booms and out the nozzles was provided by gear pumps operated by small wind-driven propellers on each plane. The boom was located beneath the lower wing on the PT 17 and inside the lower wing on the N3N. In experiments 3 and 4 the ground machine was equipped with a high-clearance, self-propelled, compressed-air, six-row, boom-type sprayer on which three nozzles were used per row to apply the spray. One nozzle was located directly above the row and the spray directed downward on the top of the cotton plant. Two other nozzles attached with flexible rubber hose were hung between the rows and sprayed horizontally into the cotton plants. T-jet nozzles were used in all experiments. Data on the spray equipment and its operation are given in table 2.

The variation in the amount of spray applied per acre—from 5 to 12 gallons—by the ground machine was due entirely to the failure of the self-powered unit to maintain a uniform speed under the variable soil conditions encountered.

Records.—Boll weevil infestation in squares and live weevil population records were made weekly in airplane experiments 1 and 2 by examining a minimum of 300 cotton squares in each treated field and the checks. The per cent of squares damaged by bollworms was obtained at the same time by observing larval feeding signs. Cotton aphid infestation, obtained only in airplane experiment 1, were made by counting the total aphids on the under side of 1 square inch of 100 leaves in each

[1] These investigations were made in cooperation with the Delta Branch of the Mississippi Agricultural Experiment Station and the U. S. Bureau of Plant Industry, Soils, and Agricultural Engineering. Certain phases were carried out under the Research and Marketing Act of 1946.

[2] The writers express their appreciation to E. W. Dunnam, who supervised the experiments, to Rudolph Strong, who conducted a substantial part of this investigation, and to W. H. Allen, manager of the Belzoni Crop Dusting Company, and L. T. Wade, Jr., manager of the South Delta Aviation Service, who applied the concentrated sprays. Presented before the meeting of the Cotton States Branch of the American Association of Economic Entomologists, Baton Rouge, La., Jan. 31 to Feb. 2, 1949.

Table 1.—Concentrated spray formulations used in experiments for control of cotton insects. (Information supplied by the formulator.)

INSECTICIDE (FIGURES IN PER CENT)	SOLVENT (FIGURES IN PER CENT)	PER CENT OF EMULSIFYING AGENT	POUNDS OF TOXICANT PER GALLON
DDT 25	Aromatic oil 73	2	2
Chlordane 46	Petroleum hydrocarbons 36.5	17.5	4
Tetraethyl pyrophosphate 40	Other phosphates 60	0	4
Gamma benzene hexachloride 26	Petroleum hydrocarbons 66	8	2.15
Toxaphene 50	Aromatic oil 25	15	5

field. The sample leaf was usually the fourth one from the top of the cotton plant. Delayed observations on insect abundance and damage were made from September 15 to October 5 on an untreated outside check field of 15 acres, 100 yards west of the field treated in experiment 1, in order to compare further the effectiveness of the insecticides.

Similar data for ground-equipment experiments 3 and 4 were obtained in the same manner and at the same interval as described for the airplane experiments, except that 100, instead of 300, squares were examined in each plot.

Yield records were obtained only in airplane experiment 1. A killing frost on October 18 prevented many of the green bolls from opening. The influence of the insecticide on yield was determined by counting all open and unopened bolls on 10 linear feet of row in eight locations in each treated field. The yields were computed from the boll count.

AIRPLANE EXPERIMENT 1.—This experiment was conducted in a field of very late cotton in which fruiting was at a peak during the progress of the experiment and at a time when most of the cotton had matured in surrounding fields. Boll weevil infestation was at a high level in these fields and weevil migration was in progress. The test field consisted of approximately 78 acres, and was divided into blocks as follows: 6 acres as an untreated check, 21 acres treated with toxaphene, 6 with gamma benzene hexachloride, 26 with chlordane, and 19 with a mixture of toxaphene and chlordane. Poison applications were begun on August 25, when the boll weevil infestation ranged from 24 to 57 per cent, and were continued at 4- to 7-day intervals until September 22, by which date 6 applications had been made. Applications were made between 3:00 and 6:30 p.m. Because of a serious threat of bollworms in the blocks treated with benzene hexachloride and chlordane, 1 pound of DDT per acre was added to these insecticides in the last 3 applications.

Tetraethyl pyrophosphate and gamma benzene hexachloride were tested for "knockout" aphid control. The aphidicides were applied on September 24 between 3:30 and 3:45 p.m. at the rate of 1.95 gallons of spray per acre in the presence of a brisk wind estimated at about 12 miles per hour and at a temperature of 80° F. Conditions were recognized as being very unfavorable for aphid control if measured by the expected performance of dust applications. Because of the unfavorable flying conditions the plane's flight varied from 5 to 15 feet above the cotton.

AIRPLANE EXPERIMENT 2.—In this experiment fields were treated individually, and were from 40 feet to one-half mile apart. The check, or untreated field, was 2 acres in size; the field treated with toxaphene, 13.8 acres; with chlordane, 13.2 acres; and with a toxaphene-chlordane mixture, 16.8 acres. Although the fields were planted at approximately the same time early in May and on soil appearing to have an equal level of fertility, there was a considerable difference in the maturity of the cotton in each. From 37 to 56 per cent of the squares

Table 2.—Data on spray equipment and its operation.

ITEM	AIRPLANE		GROUND MACHINE
	Expt. 1	Expt. 2	Expts. 3–4
Spray applied per acre (gallons)	1.95	2.50	5 to 12
Spray delivered per nozzle per minute (gallons)	.58	.62	.067
Nozzle spray pressure (p.s.i.)	30	125	65
Number of nozzles per unit	19	26	18
Speed of machine (m.p.h.)	70	80	4 to 9
Swath width (feet)	40	40	20
Average diameter of spray droplets (microns)	100	40	20

were punctured by the boll weevil at the time the poison applications were begun. Six applications were made at 5- or 6-day intervals late in the afternoon from August 17 to September 13. Because of an increase in the bollworm infestation in the field treated with chlordane, DDT was added to the last two applications at the rate of 1 pound per acre.

GROUND-EQUIPMENT EXPERIMENT 3.—Two replications of each of five treatments and a check on plots 36 rows wide and approximately one-half acre in size were made in this experiment. The cotton was fruiting heavily at the time poison applications were begun. Boll weevil migration was also in progress. The general level of infestation was high in all plots, ranging from 43 to 62 per cent of the squares punctures. Six poison applications were made at 4- to 9-day intervals from August 6 to 30. Applications were made both early in the morning and late in the afternoon. Two of them were washed off by rain within a few hours after treatment. Because of inclement weather and muddy conditions in the field, the general progress of the experiment was seriously impaired. The self-propelled machine used in applying the insecticides was not satisfactory under the prevailing conditions.

GROUND-EQUIPMENT EXPERIMENT 4.—Four treatments and a check were replicated twice on plots 12 rows wide and one-half acre in area in experiment 4. Five poison applications were made from August 13 to 26, some in the morning and others in the afternoon. One was washed off by rain in 10 hours. When the experiment was begun the cotton had passed the peak of fruiting and the general boll weevil infestation was very high, ranging from 83 to 99 per cent of the squares punctured.

RESULTS.—Data for the four experiments are summarized in table 3. All the insecticides tested in airplane experiment

Table 3.—Effectiveness of various organic insecticides applied as concentrated sprays by airplane and ground equipment for control of several cotton insects. 1948.

TREATMENTS (FIGURES IN POUNDS)	POUNDS TOXICANT PER ACRE-APPLICATION	APHIDS PER SQUARE INCH OF LEAF	PER CENT SQUARES DAMAGED BY BOLLWORMS	BOLL WEEVILS PER 100 SQUARES	AVERAGE PER CENT SQUARES PUNCTURED BY WEEVIL	POUNDS OF SEED COTTON PER ACRE
Airplane Experiment 1—Belzoni, Miss.						
Toxaphene	2.0	2.29	1.8	1.4	29	1,408
Gamma benzene hexachloride	.3[1]	.12	4.0	1.8	40	1,271
Chlordane	1.0[1]	17.4	3.9	1.4	25	1,362
Toxaphene 1.5 plus chlordane 0.3	1.8	7.0	3.9	.6	15	1,978
Check (untreated)	—	.15	6.5	9.8	85	746
Outside check (untreated)	—	—	15.3	21.3	95	—
Airplane Experiment 2—Rolling Fork, Miss.						
Toxaphene	2.0	—	.9	3.2	30	—
Chlordane	1.0[2]	—	4.1	4.0	51	—
Toxaphene 1.5 plus chlordane 0.3	1.8	—	1.0	3.0	42	—
Check (untreated)	—	—	1.1	21.0	84	—
Ground Equipment Experiment 3—Stoneville, Miss.						
Toxaphene	2.11	—	.3	7	74	—
Toxaphene 1.46 plus chlordane 0.29	1.75	—	.0	7	78	—
Chlordane	.55	—	.3	6	82	—
	.85	—	1.0	6	84	—
	.99	—	2.0	3	82	—
Check (untreated)	—	—	1.0	12	87	—
Ground Equipment Experiment 4—Stoneville, Miss.						
Toxaphene	1.96	—	—	—	82	—
Gamma benzene hexachloride	.32	—	—	—	85	—
Chlordane	1.18	—	—	—	86	—
Toxaphene 1.78 plus chlordane 0.36	2.14	—	—	—	86	—
Check (untreated)	—	—	—	—	92	—

[1] DDT added at 1 pound per acre to last 3 applications for bollworm control.
[2] DDT added at 1 pound per acre to last 2 applications for bollworm control.

Table 4.—Effectiveness of tetraethyl pyrophosphate and gamma benzene hexachloride applied as concentrated sprays by airplane for cotton aphid control. Belzoni, Miss., September 24, 1948. Spray per acre, 1.95 gallons.

TREATMENT	POUNDS TOXICANT ACRE—APPLICATION	APHIDS PER SQUARE INCH OF LEAF		
		Before Treatment	3 Days After	11 Days After
Tetraethyl pyrophosphate	0.1	4.32	0.31	0.63
Gamma benzene hexachloride	.3	2.20	1.50	1.39
Check (untreated)	—	3.17	5.29	11.65

one gave boll weevil control. Benzene hexachloride did not exert so good control as toxaphene, chlordane, or a mixture of toxaphene and chlordane. The last mixture gave slightly better control than any of the others. There was a definite trend of more effective boll weevil control with all insecticides as the season progressed and as the temperature became lower, an indication that temperature and toxicity of the organic insecticides are closely linked. Although benzene hexachloride and chlordane alone did not control the bollworm, DDT added to these insecticides did provide an effective control. All treatments showed an increase in yield over the check. The greatest increase was obtained from the toxaphene-chlordane mixture. Cotton aphids developed after the use of all insecticides except benzene hexachloride, and were most abundant in the block treated with chlordane where DDT was added to the last three applications.

Tetraethyl pyrophosphate and gamma benzene hexachloride controlled cotton aphids when applied under very adverse conditions (Table 4). Exceptionally good control was obtained from tetraethyl pyrophosphate.

In airplane experiment two, toxaphene and the toxaphene-chlordane mixture gave better boll weevil control than chlordane alone. The average per cent of squares damaged by bollworms was higher in the field treated with chlordane than in the fields treated with the other two insecticides. DDT added to chlordane in the last two applications controlled the bollworm.

The big difference between the number of live boll weevils found in the untreated and treated plots in both experiments 1 and 2 is a further indication of the effectiveness of the insecticides when applied in concentrated spray form.

Boll weevil control was very poor with all the organic insecticides tested in ground equipment experiments 3 and 4. Rains prevented poison applications from being made on schedule and may have influenced the effectiveness of the insecticides. Numerous dead weevils were found within the treated plots but, because of the great influx of weevils into the small plots, no practical control was obtained from any of the treatments. Another factor adding to the failure to obtain control may have been the necessity for greater dilution of the insecticides in order to obtain plant coverage. From 5 to 12 gallons of spray were required to treat an acre with the ground equipment and only 1.95 and 2.5 gallons were required to treat an acre with airplane equipment.

PHYTOTOXICITY.—No damage to plant tissue was observed, and at the dilutions and concentrations used the insecticides had no apparent effect on plant growth.

SUMMARY.—Several of the organic insecticides applied by airplane as concentrated sprays at the same rate of toxicant per acre as recommended in dust form for boll weevil, bollworm, or cotton aphid control gave moderate to excellent control of these insects in preliminary late-season large-scale tests at Belzoni and Rolling Fork, Miss. DDT added to gamma benzene hexachloride and to chlordane controlled bollworms in fields previously treated with these insecticides but appeared to increase the cotton aphid hazard when added to chlordane. Tetraethyl pyrophosphate and gamma benzene hexachloride at 0.1 and 0.3 pound of the technical material per acre, respectively, applied by airplane in 1.95 gallons of water controlled the cotton aphid. Tetraethyl pyrophosphate appeared to be much more toxic than gamma benzene hexachloride under the prevailing conditions. Increased yields were obtained with all

the insecticides. The toxaphene-chlordane mixture gave a higher yield than any of the other treatments.

The organic insecticides applied as sprays with ground equipment at Stoneville, Miss., failed to give any appreciable boll weevil control. Dilution of the insecticides, rain, extended intervals of application, and small plot size may have been factors contributing to the failure.

The conflicting results obtained between airplane and ground-equipment applications cannot be explained by the data available.

There was no injury to plants from any of the insecticides tested.

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Effectiveness of Different Concentrations of Parathion Alone and of Oil with Parathion to Control California Red Scale

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Parathion was the first of the new organic insecticides to show marked toxicity to a wide range of scale insects. Many workers have recorded data showing its effectiveness against species of scales. Metcalf (1949) found parathion very toxic to California red scale (*Aonidiella aurantii* (Mask.), in laboratory tests, and Cressman *et al.* (1949) reported results of field experiments indicating that parathion alone and in combination with oil was a promising material for red scale control.[1] Field studies on the control of this scale with different concentrations of parathion alone have been continued and the effectiveness of different amounts of parathion in combination with two concentrations of two weights of oil has been investigated. The results are reported in this paper.

A 25 per cent wettable powder was used in all sprays. Applications were made by commercial spray operators with conventional equipment. The methods of making the insect population counts were similar to those described by Cressman & Broadbent (1944). Counts were limited to adult female scales. Oil deposits were measured by methods described by Dawsey & Hiley (1937) and modified by Cressman (1941).

CONCENTRATION OF PARATHION ALONE. —Sprays were applied September 28, 1948 to a lemon grove heavily infested with California red scale. Concentrations of 25 per cent parathion wettable powder of 1 and 1.5 pounds per 100 gallons were applied to two plots each, and of 2 pounds per 100 gallons to four plots. Plots, arranged at random, consisted of 16 to 26 trees and were covered by 200 gallons of spray. Spreaders and a miticide were added to certain plots but there was no evidence that they influenced effectiveness against red scale.

Ten trees were selected for uniformity of size and condition in each plot and all population counts were made on these trees. The pretreatment infestation was measured on 3-inch long units of the gray wood of the branches. The average infestation per plot ranged from 4.8 to 11.0 live scales per unit. Scale infestation was increasing by the following May and posttreatment population records were made May 16 to 24. Beacuse of the high natural mortality on the gray wood, counts were made on 6-inch units of the green wood where dead scales could be eliminated by inspection. Infestation on the fruit at this time was too light to give a reliable sample of the scale population. Results are given in table 1.

[1] News Letter No. 39, Calif. Univ., Citrus Expt. Sta., Riverside, Calif., contains brief comments which indicate the possible value of parathion for controlling scale insects on citrus.

Table 1.—Results with sprays containing different concentrations of parathion.

PARATHION CONCENTRATIONS	PRE-TREATMENT INFESTATION	POST-TREATMENT INFESTATION
Lbs. 25 per cent wettable powder per 100 gal.	*Scales per unit*	*Scales per unit*
1.0	9.4	1.94
1.5	8.1	1.29
2.0	8.1	.97

The results suggest a trend toward better control with increasing concentrations, but there was a large variation between replicated plots and the differences were not statistically significant

All plots were sprayed on June 7, 1949, with the same parathion concentrations applied the preceding September, but no additives were included in the second treatment. Fruit in some of the plots had become heavily infested by fall and counts on 200 fruits in each plot, September 26–28, gave the following results:

PARATHION (POUNDS 25 PER CENT)	
WETTABLE POWDER (PER 100 GAL.)	SCALES PER FRUIT
1.0	10.4
1.5	2.7
2.0	1.1

The differences between parathion concentrations were highly significant ($P = .01$). Infestation in the plots sprayed with 2 pounds per 100 gallons was about one-tenth that in the plots sprayed with 1 pound.

An adjacent plot was sprayed in October 1948 and June 1949 with 1.8 per cent medium oil in order to afford a comparison with a standard treatment for red scale control. In September, 1949, infestation on the fruit and green twigs in the oil plot was comparable to that in the plots sprayed with 2 pounds of 25 per cent parathion, but infestation on the older wood of the branches was considerably heavier in the oil plot. A larger plot sprayed with 2 gallons of medium oil plus 0.5 pound of 25 per cent parathion per 100 gallons in October 1948 was at least as good as plots sprayed twice with 1.5 pounds of 25 per cent parathion.

In an earlier report Cressman *et al.* (1949) found no difference in the effectiveness of single applications of sprays containing 1.5, 2 and 2.5 pounds of 25 per cent parathion per 100 gallons made in the spring. Trees in those plots had been sprayed with oil the preceding fall. When the parathion sprays were applied most of the live scales present were on the branches where parathion is most effective and all treatments gave very good results. Probably spray coverage was the important factor determining survival in those experiments. The plots used in the experiments reported herein had not been sprayed for a year preceding the first parathion spray and there was a heavy infestation on all parts of the tree when the tests were started. Results were not as good as have been obtained when there was a lighter infestation on green twigs and fruit where parathion is least effective. However, the control with 2 pounds of 25 per cent parathion compared favorably with that resulting from the standard oil spray. Spring sprays of 1.5 to 2 pounds of 25 per cent parathion per 100 gallons following oil sprays in the fall have given excellent control of red scale, probably because there were few live scales on the fruit and green twigs when the parathion was applied.

OBSERVATIONS ON OTHER INSECTS.—Citrus mealybugs were generally distributed throughout the grove before the first sprays were applied. In August 1949 no mealybugs were found in the parathion plots but they persisted in the part of the grove outside of the experimental plots. This part was sprayed with 2 per cent medium oil in October 1948.

Some soft brown scale developed in the summer of 1949. Infestation was noticeably heavier in the parathion plots than in oil-sprayed trees but was controlled by parasites by the end of the summer. Results were similar in other groves where parathion was used.

Trees were heavily infested with citrus red mite previous to the September 1948. sprays. Infestation was greatly reduced by the parathion sprays, but was increasing by the following April. All parathion plots were sprayed with (1,1-bis(*p*-chlorophenoxy) methane)[1] April 21. A heavy infestation developed throughout the grove during the following summer

[1] *Neotran.*

and another spray of this material was applied September 14. In this and other experiments, control of citrus red mite by parathion has been inferior to that obtained with oil sprays.

EFFECT ON TREES.—All trees in the parathion plots were sprayed with 2 pounds of 25 per cent parathion per 100 gallons October 25, 1949. No adverse tree reactions, either acute or chronic, have been observed in these plots, which have received three applications of parathion. There was practically no leaf drop following any of the sprays. In some other groves a moderate leaf drop has followed parathion sprays. This has usually been confined to older leaves. In two groves leaf drop on lemon trees was excessive. One of these groves consisted of older trees in rather poor condition. The other contained young vigorous trees, but sprays were applied during a prolonged period of wind and very low humidity. There was no fruit drop or twig dieback in either grove, but leaf drop was considerably greater in the trees sprayed with parathion than in those sprayed with oil at the same time.

OIL PLUS PARATHION.—Preliminary experiments showed that addition of parathion wettable powder to oil sprays greatly increased their effectiveness against California red scale. They also showed that the scale kill from such combinations was about the same on all parts of the tree (Cressman *et al.* 1949). The residual effects of parathion, such as reduced reproduction of young by surviving females, and toxicity to crawlers beneath the scale covering and to young attempting to settle, suggested combining parathion with light oils which by themselves have little residual action.

Experiments were made with two concentrations of medium and of light oil with 0.5 and 1 pound of 25 per cent parathion with each. One of the oils met the specifications for a medium oil of the California State Department of Agriculture as published by Cox (1938). It was used as a proprietary emulsion containing 90 per cent oil. The other, designated as special light oil had the following characteristics:

Viscosity (100° F.)	51 sec. Saybolt
Unsulfonatable residue	91 per cent
Distillation	15 per cent at 533° F. 82 per cent at 636° F.
Specific gravity (20° C.)	0.869

It is somewhat lighter than any of the light spray oils registered in California. It was made emulsive by incorporating 0.5 per cent of glyceryl monooleate.

Sprays were applied by a commercial spray crew to lemon trees July 6, 1948. All sprays contained the alkanoamine salt of 2,4-D at the rate of 8 p.p.m., acid equivalent (Stewart & Riehl 1948). Each material was applied to two plots, each of which contained 16 or 18 trees. The location of plots was random in a block of about 350 trees.

Eight trees in each plot were marked for population counts and used for both pre- and post-treatment counts. Pre-treatment counts were made on the 3-inch units of gray wood and post-treatment counts were made on 6-inch units of green wood

Table 2.—Results of sprays of parathion with oil applied to California red scale on lemon trees. Data on oil deposits and infestations are averages of two plots.

MATERIALS PER 100 GAL.		MEDIUM OIL			SPECIAL LIGHT OIL		
			Scales per Unit			Scales per Unit	
Oil	Parathion (25 per cent wettable powder)	Oil Deposit	Pre-Treatment	Post-Treatment	Oil Deposit	Pre-Treatment	Post-Treatment
Gallons	*Pounds*	*μml./cm.²*			*μml./cm.²*		
1.0	0.5	64	2.92	1.02	68	2.80	1.18
	1.0	65	3.03	.28	56	2.36	.26
1.75	0.0	86	1.66	2.03	80[1]	1.40	1.00
	0.5	99	3.25	.48	85	3.46	.65
	1.0	91	3.21	.30	78	2.88	.22

[1] 2.0 per cent oil was used in plots sprayed with special light oil without parathion.

January 10 to 24, 1950. The data are summarized in table 2.

The results showed that all of the combinations containing parathion were better than 1.75 per cent of medium oil, which is a standard treatment for red scale control. When parathion was added, the two weights of oil were equally effective. There was a highly significant difference between the two concentrations of parathion. Increasing the parathion concentration had the greater effect at the lower oil concentration. With 1.0 pound of 25 per cent parathion in the spray, 1.0 per cent of oil was as effective as 1.75 percent. With 0.5 pound of 25 per cent parathion, the difference between 1.0 and 1.75 per cent of oil was of doubtful significance and more investigation would be required to determine if there is any difference in the effectiveness of these two combinations.

It is possible that the reason for failure to demonstrate differences between some of the higher concentrations of materials was that these combinations were sufficiently toxic to red scale that the limiting factor in control was failure to obtain complete coverage. Spray application in these experiments was no better than average for commercial work. Some spray operators apply more spray material per tree and further studies with heavier applications may show more effect from increasing the oil concentration with parathion added.

The oil deposits from sprays containing 1.75 per cent and 2.0 per cent oil indicate a tendency for the added parathion to increase the oil deposits somewhat. While not very marked in these sprays, the same effect has been noted in other experiments.

In tests in other groves, one application of 1.75 per cent of special light oil plus 1 pound of 25 per cent parathion has been as good as or better than two applications of the conventional oil sprays of light medium to heavy medium oil. One application of full dosage medium oil or heavy medium oil plus 0.5 pound of 25 per cent parathion has sometimes equalled two of the conventional oil sprays. In other cases it has not been so effective but has always been better than an equal amount of oil alone.

OBSERVATIONS ON OTHER INSECTS.—Citrus red mite infestations in these plots remained low throughout the summer and fall but by the end of February 1950 considerable numbers of mites were found in all plots. These developed at about the same rate in the different plots regardless of treatment. In other experiments also, oil plus parathion combinations have controlled citrus red mite about as well as 1.75 per cent of medium to heavy medium oil alone.

EFFECTS ON TREES.—Leaf and fruit drop following these sprays was negligible and no other adverse effects were apparent. Considerable experience in other experiments has shown that whenever oil alone caused any large drop of leaves or fruit, or twig dieback, addition of parathion increased these effects. The defoliation from addition of parathion has been more pronounced with the special light oil than with medium or heavy medium oil. Addition of 2,4-D at 6–8 p.p.m. of the alkanoamine salt or 4 p.p.m., acid equivalent, of the ester almost entirely prevented leaf drop on lemons. Inhibition of leaf drop on oranges was not as good in all cases. In some groves a noticeable number of yellow leaves has appeared on lemons after fall applications of 1.75 to 2.0 per cent of the special light oil with 1.0 pound of parathion and 2,4-D. In the work to date this has not caused any reduction in crop, but final conclusions on the safety of the full dosage of oil plus 1 pound of 25 per cent parathion should await the results of repeated application over a period of time.

EFFECT ON SPRAYMEN.—The combinations of oil and parathion applied in these tests had no adverse effect on the spraymen. Whether they are more hazardous than sprays containing wettable powders alone, especially during periods of long and continued usage, has not been determined.

SUMMARY.—Lemon trees were sprayed in September 1948, and June 1949 with 1.0, 1.5, and 2.0 pounds of 25 per cent parathion per 100 gallons. In September 1949, California red scale, *Aonidiella aurantii* (Mask.), infestation in the plots sprayed with 2.0 pounds was about one-tenth that in the plots sprayed with 1.0 pound per 100 gallons.

Parathion alone was most effective when there were few scales on green twigs and fruit.

A special light and a medium oil were equally effective when parathion was added. One per cent of oil with 0.5 pound

of 25 per cent parathion per 100 gallons was more effective than 1.75 per cent medium oil alone. Increasing the 25 per cent parathion concentration from 0.5 to 1 pound with 1 gallon of oil per 100 gallons greatly increased effectiveness against red scale. When 1 pound of 25 per cent parathion was added, 1.75 per cent of oil was no more effective than 1 per cent.

Parathion gave good control of an infestation of citrus mealybug, but was relatively ineffective against citrus red mite. Sprays of parathion with oil were at least as effective as oil alone against red mite. Infestations of soft brown scale increased in plots sprayed with parathion alone. In most cases there was no apparent injury to trees sprayed with 25 per cent parathion wettable powder alone at the rate of 1.5 to 2 pounds per 100 gallons. In two lemon groves there was excessive leaf drop, but no fruit drop or twig dieback, following parathion sprays.

Whenever oil alone caused any large drop of leaves or fruit, or dieback, addition of parathion to the oil increased the injury. Addition of 2,4-D almost entirely prevented leaf drop on lemons but control of leaf drop on oranges was not as good in all cases.

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Toxicity of Various Sulphur and Phosphorus Compounds Applied as Sprays on Spider Mites and Aphids[1]

J. C. GAINES,[2] E. E. IVY,[3] H. A. DEAN,[2] and A. L. SCALES[3]

Spider mites and cotton aphids, *Aphis gossypii* Glov., have been considered injurious pests of cotton for many years. These pests often develop to injurious numbers following applications of certain organic insecticides whenever weather conditions are favorable for their increase. This increase presumably results from the destruction of their natural enemies. The spider mite most commonly found infesting cotton in Texas and other Southwestern States was determined to be a new species of *Septanychus*, and a manuscript by Iglinsky & Gaines (1949) referred to it as this new species. Further studies, however, indicate that it is close to *Tetranychus opuntiae* Banks and is not *Septanychus* sp., as referred to previously. Cultures of both spider mites and aphids are maintained in the laboratory throughout the year.

Results of work conducted at this Station by Iglinsky & Gaines indicated that sulphur and parathion dusts were effective for the control of this spider mite. The increased use of organic insecticides applied as spray emulsions, however, has made it necessary to evaluate new as well as old liquid compounds for mite and aphid control. The work herein reported was conducted in the laboratory at College Station, Texas, for the purpose of evaluating some of the available sulphur and phosphorus compounds which might be used for mite and aphid control in a schedule of spray applications for the control of other cotton insects.

[1] Technical contribution 1329 Texas Agricultural Experiment Station in cooperation with the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture. Thanks are due A. L. Adams, L. J. Gorzycki, C. R. Jordan and W. J. Magee for assistance in conducting the tests. Samples of the compounds were furnished by the following companies: California Spray Chemical Corp., American Cyanamid Co., Geary Chemical Corp., Eastern Chemical Corp., United States Rubber Co., Stauffer Chemical Co., General Chemical Co., Sherwin-Williams Co., B. G. Pratt Co., J. M. F. Chemical Co., and E. I. Dupont de Nemours & Co.

[2] Texas Agricultural Experiment Station.

[3] U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine.

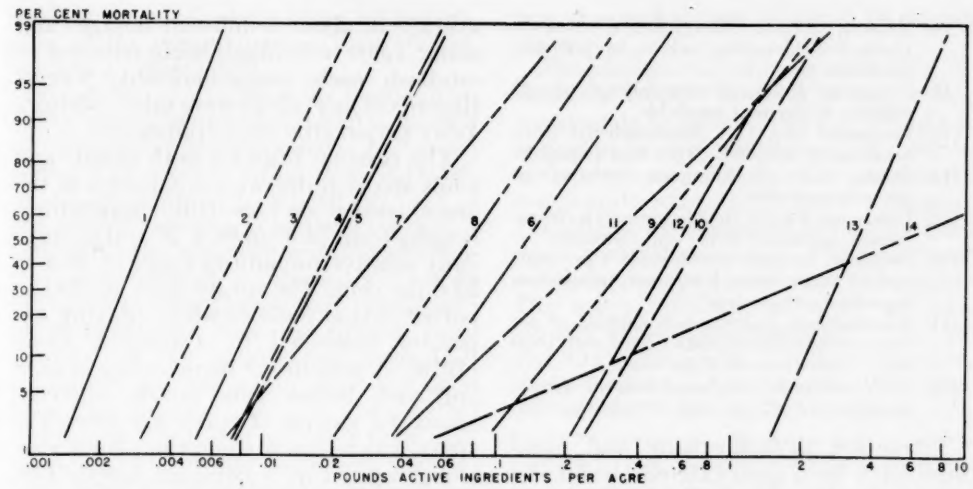

FIG. 1.—Dosage-mortality curves for the toxicity of the following insecticides to spider mites: (1) Tetraethyl pyrophosphate. (2) parathion, (3) diethoxy thiophosphoric ester of 4-methyl 7-hydroxy coumarin, (4) dialkyl nitroaryl thiophosphates, (5) tetraethyl dithionopyrophosphate, (6) octamethyl pyrophosphoramide, (7) mercurated pentaethyl triphosphate and related organic phosphoric esters, (8) 2-(p-tert. Butylphenoxy)-1-methyl-ethyl 2-chloroethyl sulfite, (9) p-chlorophenyl phenyl sulfone, (10) 2,4 dichlorophenyl ester benzene sulfonic acid, (11) 1,1-bis(p-chlorophenyl) ethanol, (12) p-chlorophenyl p-chlorobenzene sulfonate, (13) sodium polysulphide, sodium thiosulphate and (14) calcium polysulphide, calcium thiosulphate.

MATERIALS.—The sprays used in these tests were made by mixing water with the following concentrates:

(1) Tetraethyl pyrophosphate, 20 per cent emulsible
(2) Parathion, 20 per cent emulsible
(3) *Compound 838* (diethoxy thiophosphoric ester of 4-methyl 7-hydroxy coumarin) 60 per cent emulsible.
(4) *Metacide*, (dialkyl nitroaryl thiophosphates, a mixture containing 20 per cent parathion and 80 per cent methyl homolog of parathion) 33.4 per cent emulsible.
(5) *Compound 3472*, (tetraethyl dithionopyrophosphate) 25 per cent emulsible.
(6) Octamethyl pyrophosphoramide, technical.
(7) *Merthon* (mercurated pentaethyl triphosphate 10 per cent, pentaethyl triphosphate and other related organic phosphoric esters 89 per cent).

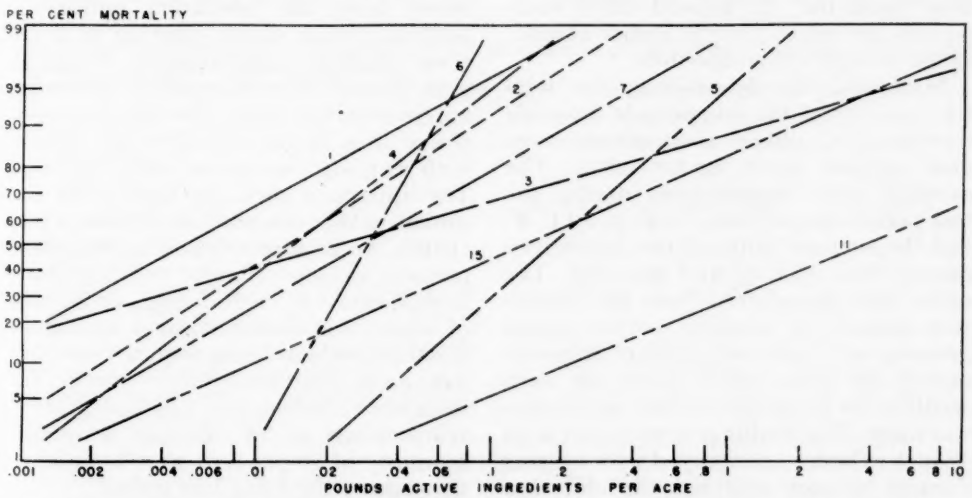

FIG. 2.—Dosage-mortality curves for the toxicity of the following insecticides to cotton aphids: (1) Tetraethyl pyrophosphate, (2) parathion, (3) diethoxy thiophosphoric ester of 4-methyl 7-hydroxy coumarin, (4) dialkyl nitroaryl thiophosphates, (5) tetraethyl dithionopyrophosphate, (6) octamethyl pyrophosphoramide, (7) mercurated pentaethyl triphosphate and related organic esters, (11) 1,1-bis(p-chlorophenyl) ethanol and (15) ethyl p-nitrophenyl benzene thionophosphonate.

(8) *Aramite*, (2-(p-tert.-Butylphenoxy)-1-methyl-ethyl 2-chloroethyl sulfite, 95 per cent emulsible.
(9) *Compound R-242*, (P-chlorophenyl phenyl sulfone) 25 per cent emulsible.
(10) *Compound 923*, (2,4 dichlorophenyl ester benzene sulfonic acid) 50 per cent emulsible.
(11) *Dimite*, (1,1-bis(p-chlorophenyl)ethanol) 25 per cent emulsible.
(12) *Compound K-6451*, (p-chlorophenyl p-chlorobenzene sulfonate) 20 per cent emulsible.
(13) *Sulfocide*, (sodium polysulphide 4 per cent, sodium thiosulphate 2 per cent, total active ingredients 42 per cent).
(14) Lime-sulphur, (calcium polysulphide 29 per cent, calcium thiosulphate 1.5 per cent, total active ingredients 30.5 per cent).
(15) *EPN*, (ethyl p-nitrophenyl benzene thionophosphonate) 27 per cent wettable powder.

Tetraethyl pyrophosphate and parathion have been used extensively for the control of mites and aphids. These compounds were included in the tests as standards for comparisons with the other materials. Other new phosphorus compounds have been evaluated recently by Magee & Gaines (1950) and Metcalf & Marsh (1949). One of these compounds, octamethyl pyrophosphoramide, has been reported by Ripper *et al.* (1950) and Ivy *et al.* (1950) to be a systemic poison exhibiting a long residual action.

With the exception of the polysulphides, the sulphur compounds have been developed recently by several commercial companies. Some are considered experimental compounds while others have been marketed. In general these compounds are not so toxic to higher animals as the phosphorus compounds.

METHODS.—In determining the relative toxicity of the compounds, miscible concentrates applied as emulsions were used against adult spider mites. The average daily temperatures during the test period ranged from 75.5° to 80.1° F. and the average daily relative humidities ranged from 45.2 to 61.1 per cent. The mites were transferred from the laboratory culture to seedling cotton plants growing in a nutrient solution. Approximately 25 mites were placed on each seedling 24 hours before the application was made. The seedlings were placed in an 18 inch cylinder and sprayed with varying dosages of each material. A solid cone nozzle delivering approximately 8 gallons of spray per acre was used. This apparatus used for the mite tests was described by Gaines & Dean (1949). Five replicate plants were sprayed at each dosage, an average of about 8 different dosages and about 1,000 individuals being required to establish each dosage-mortality curve. Records of mortalities were taken during a 4-day period after each application.

The residual tests for both aphids and adult spider mites were conducted in the greenhouse at average daily temperatures ranging from 72.6° to 98.4° F. and average daily relative humidities range of 45.6 to 92.0 per cent. The sprays were applied to potted cotton plants with a spraying apparatus described by Gaines & Dean (1950). A pressure of 60 pounds was used and each hollow cone nozzle delivered about 2.5 gallons of spray per acre. The spray was released while the plants were being rotated at a constant speed. This method compares favorably with a tractor spray machine operated in the field. Plants heavily infested with either spider mites or aphids were treated and allowed to remain in a section of the greenhouse where other plants were infested. The treated plants were subject to reinfestation throughout the test period. Four potted plants were treated with each material, and records were obtained on the mortality of either mites or aphids on 2 leaves per plant.

The relative toxicity of most of the compounds, miscible concentrates applied as emulsions, was also determined against cotton aphids. The aphids were transferred from the laboratory culture to seedling cotton plants growing in a nutrient solution. Approximately 50 aphids were placed on each seedling before the application was made. The seedlings were placed in a 12-inch cylinder and sprayed with varying dosages of each material. The apparatus used for these tests was similar to that described by Parencia *et al.* (1946). An atomizer delivering 22 gallons per acre was used. Twelve replicate plants were sprayed at each dosage, an average of about 4 different dosages and about 2,500 individuals being required to establish each dosage-mortality curve. The tests were conducted in a cabinet where a temperature of 75° F. and a relative humidity of 70 per cent were maintained throughout the 3-day test period.

A check or untreated replicate was included with each series of tests. All mortality percentages were calculated by Abbott's formula. The percentages of mortality were transformed to probits

and the dosages to logarithms for calculation of the dosage-mortality curves, according to Bliss (1938).

RESULTS.—In figure 1 the dosage-mortality curves show the relative toxicity of the several compounds to spider mites. The median lethal dose (LD 50), or dosage required to kill 50 per cent of the individuals, and regression coefficient indicating the slope of the dosage-mortality curve for each compound are shown in table 1. The phosphorus compounds were more toxic than the sulphur compounds. Of the phosphorus compounds tetraethyl pyrophosphate was the most toxic, as indicated by the low median lethal dose. Parathion was more toxic than metacide. The sulfite compound (8) was more toxic to mites than the sulfone, sulfonate, sulfonic acid or the polysulphides.

The results of the residual toxicity tests to mites are shown in table 2. The toxicity of compounds (1), (4) and (5) decreased within a 7-day period whereas compounds (2) and (7) decreased within a 10-day period. With the exception of compounds (3) and (6), the phosphorus compounds did not prove toxic longer than 10 days. Compound (6) (octamethyl pyrophosphoramide) was highly effective throughout the 14-day period, an indication of either residual or systemic action. In general the sulphur compounds were toxic throughout the 14-day period. The polysulphides were comparatively ineffective at a high dosage of about 16 pounds per acre.

The dosage-mortality curves (Fig. 2) show the relative toxicity of the several compounds to aphids. The LD 50, LD 90 and slope of the dosage-mortality curves are shown in table 3. The phosphorus compounds were highly toxic to aphids. Tetraethyl pyrophosphate was the most toxic compound tested. Parathion and metacide were equally toxic to aphids. The other materials in order of their toxicity were compounds (3), (7), (6), (15) and (5). Compound (11) was comparatively ineffective for aphid control. Since the sulphur compounds did not prove toxic to aphids no attempt was made to establish the dosage-mortality relationship. Compounds (8), (9) and (10) applied at 6.4 pounds per acre failed to give 50 per cent mortality.

The results of residual toxicity tests to aphids are shown in table 4. In general the results were the same as those obtained with the same compounds against mites. The toxicity of compounds (1), (3), (4), (5) and (7) decreased within a 7-day period and compound (2) decreased within a 10-day period. Octamethyl pyrophosphoramide was again highly effective throughout the 14-day period, another indication of its systemic or residual action. Compounds (11) and (12) applied at the rate of 1.8 pounds per acre gave 80 and 62 per cent control, respectively, the first day

Table 1.—Dosage-mortality relationship of several compounds against spider mites evaluated four days after application (laboratory tests).

COMPOUND	POUNDS OF ACTIVE INGREDIENT PER ACRE REQUIRED TO GIVE		SLOPE OF LINE
	50% Mortality	90% Mortality	
(1) Tetraethyl Pyrophosphate	0.003	0.005	7.88
(2) Parathion	.008	.015	4.31
(3) Diethyl thiophosphoric ester of 4-methyl 7-hydroxy coumarin	.012	.022	5.00
(4) Dialkyl nitroaryl thiophosphates	.021	.038	4.88
(5) Tetraethyl dithionopyrophosphate	.022	.041	4.94
(6) Octamethyl pyrophosphoramide	.139	.321	3.52
(7) Mercurated pentaethyl triphosphate and related organic phosphoric esters	.035	.085	3.42
(8) 2-(p-tert.-Butylphenoxy)-1-methyl-ethyl 2-chloroethyl sulfite	.078	.170	3.76
(9) p-chlorophenyl phenyl sulfone	.425	1.024	3.31
(10) 2,4 dichlorophenyl ester benzene sulfonic acid	.754	1.510	4.24
(11) 1,1-bis(p-chlorophenyl) ethanol	.372	1.029	2.90
(12) p-chlorophenyl p-chlorobenzene sulfonate	.605	1.213	4.24
(13) Sodium polysulphide, sodium thiosulphate	3.522	5.889	5.76
(14) Calcium polysulphide, thiosulphate	5.340	62.600	1.20

Table 2.—Comparative effectiveness of several compounds against spider mites (laboratory tests).

Compound	Pounds of Active Ingredients per Acre	Per Cent Mortality on Indicated Days After Application				
		1	3	7	10	14
(1) Tetraethyl pyrophosphate	0.05	100	97	62	0	0
(2) Parathion	.06	99	100	96	67	49
(3) Diethyl thiophosphoric ester of 4-methyl 7-hydroxy coumarin	.06	84	98	91	95	98
(4) Dialkyl nitroaryl thiophosphates	.06	99	98	86	0	0
(5) Tetraethyl dithionopyrophosphate	.06	96	97	27	33	53
(6) Octamethyl pyrophosphoramide	.5	93	100	90	100	100
(7) Mercurated pentaethyl triphosphate and related organic phosphoric esters	.06	94	97	96	81	74
(8) 2-(p-tert.-Butylphenoxy)-1-methyl-ethyl 2-chloroethyl sulfite	.25	94	99	98	78	82
(9) P-chlorophenyl phenyl sulfone	1.0	94	84	65	86	82
(10) 2,4 dichlorophenyl ester benzene sulfonic acid	1.8	94	99	100	98	93
(11) 1,1-bis(p-chlorophenyl) ethanol	1.0	70	94	97	100	99
(12) P-chlorophenyl p-chlorobenzene sulfonate[1]	1.8	61	99	100	99	91
(13) Sodium polysulphide, sodium thiosulphate	15.9	56	40	26	20	29
(14) Calcium polysulphide, calcium thiosulphate	15.9	64	51	72	44	15

[1] Insecticide injured the plants.

Table 3.—Dosage-mortality relationship of several compounds against cotton aphids evaluated three days after application. (laboratory tests).

Compound	Pounds of Active Ingredient per Acre Required to Give		Slope of Line
	50% Mortality	90% Mortality	
(1) Tetraethyl pyrophosphate	0.006	0.045	1.80
(2) Parathion	.016	.090	1.82
(3) Diethoxy thiophosphoric ester of 4-methyl 7-hydroxy coumarin	.027	1.580	.73
(4) Dialkyl nitroaryl thiophosphates	.017	.066	2.27
(5) Tetraethyl dithionopyrophosphate	.195	.684	2.89
(6) Octamethyl pyrophosphoramide	.031	.062	3.30
(7) Mercurated pentaethyl triphosphate and related organic phosphoric esters	.029	.238	1.42
(11) 1,1-bis(p-chlorophenyl) ethanol	4.000	49.360	1.72
(15) Ethyl p-nitrophenyl benzene thionophosphonate	.156	1.984	1.66

but the toxicity of both compounds decreased by the third day. Compounds (8), (9) and (10) were ineffective for aphid control. Compound (12) applied at 1.8 pounds per acre was toxic to the plant.

SUMMARY.—The following compounds are listed in order of their effectiveness for both spider mite and aphid control: Tetraethyl pyrophosphate, parathion, diethoxy-thiophosphoric ester of 4-methyl 7-hydroxy coumarin, dialkyl nitroaryl thiophosphates, tetraethyl dithionopyrophosphate, mercurated pentaethyl triphosphate and octamethyl pyrophosphoramide. The sulphur compounds were not effective for aphid control. The following sulphur compounds are listed in order of their effectiveness for spider mite control: 2-(p-tert-butylphenoxy)-1-methyl-ethyl 2-chloroethyl sulfite), p-chlorophenyl phenylsulfone, p-chlorophenyl p-chlorobenzene sulfonate, 2,4 dichlorophenyl ester benzene sulfonic acid and the polysulphide compounds. The 1,1-bis(p-chlorophenyl) ethanol was not as toxic to spider mites as 2-(p-tert-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite, but was more toxic than the sulfone or sulfonic acid compounds. Octamethyl pyrophosphoramide remained highly toxic during the 14-day period an indication of either residual or systemic action. The residual

Table 4.—Comparative effectiveness of several compounds against cotton aphids. (laboratory tests).

Compound	Pounds of Active Ingredients per Acre	Per Cent Mortality on Indicated Days After Application				
		1	3	7	10	14
(1) Tetraethyl pyrophosphate	0.03	97	90	58	0	0
(2) Parathion	.06	95	96	95	85	26
(3) Diethoxy thiophosphoric ester of 4-methyl 7-hydroxy coumarin	.2	69	80	14	25	41
(4) Dialkyl nitroaryl thiophosphates	.06	99	98	93	59	0
(5) Tetraethyl dithionopyrophosphate	.2	48	15	38	0	0
(6) Octamethyl pyrophosphoramide	1.0	98	100	100	100	100
(7) Mercurated pentaethyl triphosphate and related organic phosphoric esters	.2	83	72	63	30	0
(8) 2-(p-tert.-Butylphenoxy)-1-methyl-ethyl 2-chloroethyl sulfite	1.0	0	0	0	0	0
(9) P-chlorophenyl phenyl sulfone	1.8	0	0	47	66	1
(10) 2,4 dichlorophenyl ester benzene sulfonic acid	1.8	0	0	0	0	0
(11) 1,1-bis(p-chlorophenyl ethanol)	1.8	80	37	36	0	0
(12) P-chlorophenyl p-chlorobenzene sulfonate[1]	1.8	62	56	—	—	—
(14) Calcium polysulphide, calcium thiosulphate	15.9	0	0	13	0	0

[1] Insecticide injured plants.

toxicity of the other phosphorus compounds decreased within a 10-day period. The sulfite, sulfone, sulfonic acid compounds and diethoxy thiophosphoric ester of coumarin remained toxic during the 14-day period.

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Z. P. METCALF RETIRES

Z. P. Metcalf, since 1912 head of the Department of Zoology and Entomology at the North Carolina State College of Agriculture and Engineering and the Agricultural Experiment Station of the University of North Carolina, and since 1940 Associate Dean of the Graduate School, University of North Carolina, has retired from administrative duties and will devote his entire time to his work on cataloguing the Homoptera of the World and taxonomic work on the Homoptera. The section of Entomology will be headed by Dr. Clyde F. Smith and the section of Zoology by Dr. Fred S. Barkalow.

Translocation of Octamethyl Pyrophosphoramide by the Cotton Plant and Toxicity of Treated Plants to Cotton Insects and a Spider Mite[1]

E. E. Ivy, Wm. Iglinsky, Jr., and C. F. Rainwater, *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*[2]

Insecticidal activity of octamethyl pyrophosphoramide was reported by Schrader (1947). Formulations containing this compound are now being sold in England. The compound is reported to be an especially effective aphidicide.

Other workers have found that parathion, which is also a phosphate-containing insecticide, is absorbed and translocated by plants to which it is applied. Questel & Connin (1947) found that parathion was absorbed by the roots of corn plants, and that the stalks and leaves of treated plants were toxic to larvae of the European corn borer. Granger and Leiby (1949) reported that, after the translocation of parathion, plants were toxic to several species of aphids and to Mexican bean beetle larvae, but not to the two-spotted spider mite.

In tests at College Station, Texas, during 1948, parathion failed to control the cotton aphid, the boll weevil, the bollworm, and a spider mite, when applied to the soil at dosages ranging from 0.5 to 2 pounds per acre.

Laboratory, greenhouse, and field tests were conducted at College Station during 1949, to determine whether octamethyl pyrophosphoramide is absorbed and translocated by cotton plants, when applied to control cotton insects and a spider mite. The material used in these tests was obtained from the Division of Insecticide Investigations of this Bureau. The technical material is a liquid and is readily soluble in water. It was used in water solution and in dusts made by impregnating Attaclay with acetone solutions of the compound, evaporating the acetone, and grinding in a ball mill.

Laboratory Experiments with Nutrient Solution.—Experiment 1 was designed to determine the effectiveness of octamethyl pyrophosphoramide against a spider mite and the cotton aphid, *Aphis gossypii* Glov., when the compound was placed in a complete nutrient solution in which seedling cotton plants were growing. Preliminary tests had shown that a concentration of 153.6 parts of octamethyl pyrophosphoramide to 1 million parts of nutrient solution was entirely effective against both spider mites and aphids. In this experiment 1.2 to 76.8 parts of the toxicant per million parts of solution were used. On April 12 young plants were placed in vials of nutrient solution containing the toxicant. Two weeks later they were removed, rinsed, and placed in vials containing nutrient solution but no toxicant. Two plants from each treatment were infested with cotton aphids, and counts of living aphids were made for 3 days. Two plants from each treatment were infested with spider mites, and similar records were made.

The results of the experiment are shown in table 1. All aphids were killed at a concentration of 38.4 p.p.m., and all spider mites at 9.6 p.p.m.

Greenhouse Experiments with Soil Treatments.—In experiment 2 various concentrations of cotamethyl pyrophosphoramide in water in water solution were applied to the soil in which cotton plants were growing. The plants were approximately 12 inches tall and 2 months old. They were growing in glazed 2-gallon jars of 7.75 inch diameter at the top. Dosages were from 1 to 256 pounds of the toxicant per acre. Fifteen plants were used for the untreated check and for each of the four lowest dosages, and three each for the five highest dosages. The dose for each jar was diluted with 100 milliliters of water, and this was poured uniformly over the soil surface. Care was taken not to get any of the solution on the leaves.

A heavy infestation of aphids and a light infestation of spider mites were on the plants at the time the treatments were made. Counts of aphids and mites were made just before treatment and at intervals during the tests. These counts were made on two to five leaves per plant.

Records made in the experiment are

[1] Report of a study made under the Research and Marketing Act of 1946.

[2] In cooperation with the Texas Agr. Expt. Stat.

Table 1.—Effect of cotton aphids and spider mites when placed on seedling cotton plants which had been growing for 2 weeks in nutrient solution containing octamethyl pyrophosphoramide. Experiment 1.

Concentration of Octamethyl Pyrophosphoramide (p.p.m.)	Number of Live Spider Mites per Plant				Number of Live Cotton Aphids per Plant			
	Placed on Plants	After 1 Day	After 2 Days	After 3 Days	Placed on Plants	After 1 Day	After 2 Days	After 3 Days
76.8	25	0	0	0	53	8	0	0
38.4	25	0	0	0	43	7	2	0
19.2	25	0	0	0	43	23	20	20
9.6	25	8	0	0	44	33	17	16
4.8	25	19	6	1	46	32	27	20
2.4	25	24	15	8	52	32	20	30
1.2	—	—	—	—	41	50	50	69
Untreated check	25	24	23	21	49	57	58	64

shown in table 2. The higher dosages were extremely effective against both spider mites and aphids within 2 to 3 days. Later counts confirmed these early records. About 2 days prior to the count, which was made on the twenty-fifth day after treatment, the plants were uniformly reinfested with aphids and spider mites. Counts made 25 and 50 days after treatment indicated that dosages of 8 pounds per acre or higher were still extremely effective.

Experiment 3 was conducted to determine how long octamethyl pyrophosphoramide remains effective after it is applied to the soil. Dosages used in the experiment were 25, 50, and 75 pounds of the toxicant per acre. Six plants received each treatment, and counts were made on two leaves from each plant from February 22, the date the experiment was started, until October 24. The spider mite was used as the test species. As is shown in table 3, the plants were lightly infested at the time the experiment was begun. All concentrations were extremely effective for 53 days, and then gradually lost their effectiveness. However, for 223 days after the single soil application all the treated plants had mite populations much lower than those of the untreated checks.

In order to maintain a high infestation of spider mites, all the plants were reinfested 6 to 10 days before counts were to be made. On the count made 246 days after the soil treatment, the population on the check plants was for the first time lower than that on plants in treated soil. The check plants were in very poor condition, owing to prolonged mite injury, and it was thought that possibly the low count was due to the condition of the plants. All the plants were then trimmed back to a few inches from the ground. After new leaves appeared mites were placed on the plants, and a final record was made 272 days after the soil treatment. This record indicated that little if any of the toxicant was present in the new plant growth.

GREENHOUSE EXPERIMENT TO COM-

Table 2.—Infestations of spider mites and cotton aphids as affected by various dosages of octamethyl pyrophosphoramide in water solution applied to soil in which infested cotton plants were growing. Experiment 2.

Octamethyl Pyrophosphoramide, Pounds per Acre	Spider Mites, Number per Leaf					Cotton Aphids, Number per Leaf						
	Before Treatment	After Treatment				Before Treatment	After Treatment					
		3 Days	9 Days	18 Days	25 Days[1]		2 Days	6 Days	10 Days	17 Days	25 Days	50 Days
1	35	23	8	8	16	61	84	89	23	11	12	31
2	28	16	4	10	16	61	118	115	28	15	11	12
4	29	15	2	2	5	58	64	18	8	14	3	22
8	44	14	0	1	3	66	143	27	22	13	6	4
16	25	3	0	0	0	54	103	25	1	6	8	0
32	40	4	0	0	0	63	63	15	0	2	1	0
64	30	0	0	0	0	68	14	1	0	1	0	0
128	26	0	0	0	0	78	2	0	0	0	0	0
256	23	0	0	0	1	53	0	0	0	0	0	0
Untreated check	26	27	5	6	22	61	163	155	23	6	23	66

[1] Plants were reinfested with both aphids and spider mites prior to this count.

Table 3.—Length of time octamethyl pyrophosphoramide remained effective against spider mites on cotton plants when applied in water solution to soil in which the plants were growing. Experiment 3.

Octamethyl Pyrophosphoramide, Pounds per Acre	Number of Spider Mites per Leaf											
	After Treatment											
	Before Treatment	7 Days	14 Days	28 Days	53 Days	67 Days[1]	80 Days	95 Days[1]	114 Days	223 Days[1]	246 Days[1]	272 Days[1,2]
25	39	3	1	1	1	43	9	28	7	19	23	130
50	19	1	0	0	0	19	0	4	7	19	43	105
75	17	0	0	0	0	0	0	4	1	19	28	110
Untreated check	63	43	155	166	20	113	150	89	49	51	11	161

[1] Plants were reinfested 6 to 10 days before these records were made.
[2] Plants were cut back 16 days before reinfestation.

PARE SPRAY AND SOIL TREATMENTS.—Preliminary tests indicated that octamethyl pyrophosphoramide was effective against cotton aphids and spider mites when applied as a spray to cotton plants. Treated leaves remained toxic for several weeks, and there were indications that somewhat lower dosages were required than for the soil treatments.

Experiment 4 was a comparison of sprays and soil treatments. Soil treatments of 4, 8, and 16 pounds of toxicant per acre were made in the same manner as described above, with cotton plants that had been growing in 1-gallon cans for 2 months. Spray treatments were made in a spraying cylinder. The rates of spray application were 1, 2, and 4 pounds of toxicant in 3 gallons of water per acre. Six plants received each treatment.

The plants were free of insects and mites when treated, and were all infested uniformly with spider mites 5 days after the treatments. Counts of live mites made at various intervals are shown in table 4.

It will be noted that the sprays became effective much more quickly than did the soil treatments. Each of the sprays continued to be effective for 26 days and at this time the soil treatments had also taken effect. The spray treatment at 4 pounds per acre was much more effective than the corresponding soil treatment, however. Fifty-one days after the treatments the sprayed plants were no longer effective against the spider mite. The soil treatment at 4 pounds per acre was also ineffective, but those at 8 and 16 pounds were still highly effective. These results are in line with experiment 2, in which 8 pounds per acre were required for prolonged control.

Table 4.—Comparison of spray and soil treatments with octamethyl pyrophosphoramide against spider mites on cotton plants. Experiment 4.

Treatment	Pounds of Toxicant per Acre	Number of Spider Mites per Leaf at Intervals after Treatment		
		10 Days[1]	26 Days	51 Days
Spray	1	43	47	64
	2	6	11	103
	4	9	10	145
Soil	4	101	95	98
	8	96	37	9
	16	72	3	1
Untreated check	—	102	176	60

[1] Mites were placed on plants 5 days after treatment.

GREENHOUSE EXPERIMENT WITH SEED TREATMENT.—Preliminary tests were started on April 15 to determine whether plants grown from seed soaked in water solutions of octamethyl pyrophosphoramide are toxic to insects and mites. Seed was soaked for 2 hours in 2, 1, and 0.5 per cent solutions, drained, and immediately planted in sand. Plants were pulled from the sand flats 6, 13, and 35 days later and infested with spider mites and aphids. A complete kill of both species was obtained with all treatments.

Another preliminary seed-treatment test was begun on April 24. In this text cottonseed was treated with octamethyl pyrophosphoramide dusts. These dusts were prepared by impregnating Attaclay with an acetone solution of the toxicant, drying, and then grinding in a ball mill. Seed was mixed with the dusts, separated from the excess dust, and planted in sand. Six days later plants were pulled from the various flats, placed in nutrient solution, and infested with mites and aphids. After 5 days the mortality (per cent) of mites and aphids caused by the various concentrations (per cent) was as follows:

MITES		APHIDS	
Mortality	Dust Concentration	Mortality	Dust Concentration
21	0.2	91	3.2
56	0.4	72	1.6
74	0.8	8	.8
94	1.6	None	below .8
89	3.2		

On May 26, 33 days from the date of seed treatment, plants, were again pulled from the seed flats and infested with mites and aphids as before. At this time the insecticidal activity of the compound seemed to have been dissipated, as no kill of either species occurred on these plants.

Experiment 5 was designed to compare two methods of seed treatment. Seed was soaked for 2 hours in known volumes of water solutions containing 1, 0.5, and 0.25 per cent of octamethyl pyrophosphoramide. Then the excess liquid was drained from the seed and measured to determine the dosage. In the second method dust treatments were made as described in the preliminary tests, except that various amounts of a 25 per cent dust were used. The treated seed was then planted in sand.

The results of this experiment are shown in table 5. The amount of toxicant per acre was calculated on the basis of a seeding rate of 20 pounds per acre. Spider mites were used as the test species. Water solutions appeared to be much more effective than dusts. The two highest dosages of the water solution gave almost complete kill of both adults and nymphs. The 0.04 pound dosage was effective against adults, but did not kill the nymphs. All the dust treatments were effective against adults, but ineffective against nymphs.

FIELD-PLOT EXPERIMENTS.—Field-plot experiments to determine translocation of octamethyl pyrophosphoramide were conducted in the summer of 1949.

Experiment 6, conducted on five 0.001-acre plots, was started on August 18. Cotton in the plots had been planted for 4 weeks and had six leaves. Various concentrations of octamethyl pyrophosphoramide were sprayed uniformly over the cotton plants and the soil surface to give dosages of 1, 2, 4, and 8 pounds of toxicant per acre. In each plot the rate of application was 100 gallons per acre.

At the time the experiment was started a light infestation of aphids was present. Counts were made just before treatment and at intervals thereafter. These records are shown in table 6. It will be noted that by the fifth day after the treatment each dosage of octamethyl pyrophosphoramide was highly effective. At this time each of the plots was reinfested with aphids. All the dosages were also effective against this reinfestation. The plants were next infested with spider mites. Thirty-three

Table 5.—Comparison of two methods of treating seed with octamethyl pyrophosphoramide for control of spider mites on cotton plants. Experiment 5.

SEED TREATMENT	POUNDS OF TOXICANT PER ACRE[1]	NUMBER OF SPIDER MITES PER PLANT AT VARIOUS INTERVALS AFTER TREATMENT[2]						
		8 Days	9 Days	10 Days	11 Days		12 Days	
		Adults	Adults	Adults	Adults	Nymphs	Adults	Nymphs
Soaked in a water solution of octamethyl pyrophosphoramide	.18	0	0	0	0	4	0	0
	.08	0	0	0	0	0	0	1
	.04	18	16	13	3	76	1	160
Covered with a dust containing 25 per cent of octamethyl pyrophosphoramide on Attaclay	.2	19	10	6	0	103	0	112
	.1	20	20	18	11	49	6	130
	.05	18	20	18	4	68	4	187
Check, water	—	20	20	19		59	14	96
Check, Attaclay	—	20	18	18	16	60	15	120

[1] When the rate of seeding was 20 pounds per acre.
[2] Twenty adult mites were placed on each plant 7 days after seed treatment.

Table 6.—Infestations of cotton aphids and spider mites as affected by various dosages of octamethyl pyrophosphoramide in solution sprayed uniformly over field plots of cotton plants in the 6-leaf stage. Experiment 6.

Octamethyl Pyrophosphoramide, Pounds per Acre	Cotton Aphids per Plot							Adult Mites per Plot	
		After Treatment						After Treatment	
	Before Treatment	1 Day	2 Days	3 Days	5 Days	8 Days[1]	12 Days	23 Days[2]	33 Days
1	133	102	14	10	14	488	505	487	148
2	206	116	9	2	5	418	209	130	73
4	114	94	22	17	1	185	121	252	39
8	121	0	0	1	0	137	11	18	0
Untreated check	215	199	155	135	106	1040	2416	442	606

[1] Plants were reinfested 3 days prior to this record.
[2] Plants were infested 2 days prior to this record.

days after the treatments, reductions in the spider mite population were 76, 88, 94, and 100 per cent from the 1-, 2-, 4-, and 8-pound dosages, respectively.

Experiment 7, the second field-plot experiment, was begun on August 22. This experiment was conducted on ten 0.001-acre plots. Cottonseed was soaked for 4 hours in 2, 1, 0.5, and 0.25 per cent solutions of octamethyl pyrophosphoramide, drained, and planted in 40-inch rows at the rate of 50 pounds of seed per acre. Delinted and nondelinted seed was treated with each concentration of toxicant. The dosage was calculated by determining the amount of toxicant taken up by the seed planted in each plot.

As soon as the plants were above the ground, each plot was uniformly infested with aphids. Counts made at intervals on 10 plants selected at random in each plot are shown in table 7. Each dosage kept the aphid population at a very low level for 36 days.

TESTS WITH VARIOUS INSECTS.—Metcalf & March (1949) found that octamethyl pyrophosphoramide was ineffective against the honey bee, American cockroach, and house fly. Although octamethyl pyrophosphoramide was highly specific against the cotton aphid and the spider mite translocation tests conducted against several other species of cotton insects show it to have little or no effect on them, as reported below.

Boll Weevil.—Adults of the boll weevil, *Anthonomus grandis* Boh., were not killed when caged on plants from seed given the various treatments in experiment 5, or when caged on plants in each of the treated plots in experiment 6. It was thought that possibly the tissue of treated plants would be toxic to developing larvae inside the squares. Accordingly, collections of punctured squares were made from the various plots in experiment 5, on five separate dates, approximately 1 week apart. Two collections were made

Table 7.—Effect of soaking cottonseed for 4 hours in water solutions of octamethyl pyrophosphoramide on aphid infestation of cotton plants. Experiment 7.

Octamethyl Pyrophosphoramide		Condition of Seed	Aphids on 10 Plants at Various Intervals after Seed Treatment			
Concentration, per Cent	Pounds per Acre		17 Days	22 Days	29 Days	36 Days
2	0.62	De-linted	7	0	0	0
	.5	Not de-linted	3	6	0	0
1	.3	De-linted	0	11	3	0
	.25	Not de-linted	3	5	8	5
0.5	.13	De-linted	12	5	6	18
	.12	Not de-linted	23	12	12	6
0.25	.06	De-linted	24	10	7	22
	.05	Not de-linted	45	42	19	4
Untreated check	—	De-linted	476	431	237	363
Untreated check	—	Not de-linted	332	352	518	806

from the plots of experiment 6, and over a period of 2 months all the punctured squares were removed from plants in experiments 2 and 3. Adult weevils were reared on plants from seed given each of the various treatments, and the weevil emergence from squares on treated plants was about the same as from squares on the untreated plants.

Bollworm.—In laboratory tests octamethyl pyrophosphoramide failed to kill first- and second-instar larvae of the bollworm, *Heliothis armigera* (Hbn.), placed on plants growing in nutrient solutions containing 0.125 per cent of the toxicant. This concentration was 32 times as high as was required for 100 per cent kill of aphids or mites. Third-instar bollworm larvae were not killed when caged on plants in the treated plots of experiment 6. These tests were begun on October 8, 51 days after the insecticidal treatments.

Cotton Leafworm.—First-instar larvae of the cotton leafworm, *Alabama argillacea* (Hbn.), were not killed when they were placed on leaves removed from plants in the treatments in experiment 1, in which the dosage ranged from 1 to 256 pounds per acre. A dust containing 25 per cent of octamethyl pyrophosphoramide in attaclay likewise failed to control cotton leafworms when it was applied at the rate of 10 pounds per acre.

Cotton Fleahopper.—Adults of the cotton fleahopper, *Psallus seriatus* (Reut.), were caged on plants 17, 24, and 35 days after the treatment in experiment 6, where the rate of application ranged from 1 to 8 pounds of toxicant per acre. Fleahoppers survived for 5 days in all cases. On October 11, in experiment 2, plants were caged and infested with fleahopper adults. The compound appeared to kill fleahoppers slowly at the higher dosages. There was complete mortality 5 days after exposure to the plants at all dosages of 16 pounds per acre or higher. At dosages of 1 to 8 pounds per acre the survival was about the same as in the untreated checks.

Miscellaneous Insects.—First- and second-instar larvae of the salt-marsh caterpillar, *Estigmene acrea* (Drury), were not killed when placed on plants growing in nutrient solution containing 0.125 per cent of octamethyl pyrophosphoramide. Newly hatched grasshopper nymphs and adults of the leafhopper *Scaphytopius irroratus* (Van D.), were not killed when placed on these plants. Whiteflies, *Trialeurodes abutilonea* (Hald.), built up on treated plants in the greenhouse. An infestation of garden webworm severely damaged plants in each plot of experiments 6 and 7 during October.

OBSERVATION ON PHYTOTOXICITY.—In the nutrient-solution experiment cotton plants were killed by solutions containing 0.5 per cent of octamethyl pyrophosphoramide, and 0.25 per cent solutions stunted the plants. No injury was observed with a 0.125 per cent concentration.

In the soil tests with potted plants the two highest dosages, 256 and 128 pounds per acre, caused leaf shed and stunting, and it appeared for a time that these plants would be killed. However, new leaves were produced, and the plants partially overcame the injury. No injury could be detected at the lower dosages. Some reduction in stand occurred in both the laboratory and field-plot seed-treatment experiments with the 2 per cent octamethyl pyrophosphoramide solution. Seed treatment with the lower dosages did not appear to cause injury.

TESTS ON MODE OF ACTION OF OCTAMETHYL PYROPHOSPHORAMIDE.—Eaton (1949) reported that after treatment with water solutions of certain phosphorus compounds willow shoots gave off fumes that were highly toxic to brassy willow beetles *Phyllodecta vitellinae* (L.). Fumigant activity of the plant tissue was reported to be one hundred times as great as comparable experiments with aqueous solutions would indicate.

Several tests were conducted to determine whether anything of this nature occurred in cotton plants treated with octamethyl pyrophosphoramide.

In the first test a plant was sprayed with a water solution of the compound at the rate of 4 pounds of toxicant per acre. Three weeks later 100 per cent kill of spider mites had occurred on this plant. The treated plant was then enclosed with an untreated infested plant inside a sealed fumigation chamber. The chamber was placed in an incubator maintained at 90° F. No kill of spider mites occurred during a 2-day test period.

In the second test the dosage was stepped up to 160 pounds of octamethyl pyrophosphoramide per acre. This test was conducted in the same way, except that the temperature varied between 82°

and 88° F. No kill was obtained during a 2-day test period.

Since it appeared from these two tests that the toxic principle in treated plants did not kill by fumigation, a third test was conducted to determine whether octamethyl pyrophosphoramide undergoes any increase in toxicity as a consequence of its translocation by the cotton plant. Small cotton plants were placed in 200 milliliters of nutrient solution containing 1 part of octamethyl pyrophosphoramide to 800 parts of water. The plants were allowed to grow in the solution for 2 weeks. At this time the plants had taken up practically all the liquid in the container, and it was assumed that they had absorbed all the toxicant they possibly could from the solution.

The plants were then removed from the solution, thoroughly washed, and ground in a mortar. The ground plants were filtered, and the filtrate was diluted to 200 milliliters, the same volume as was used originally. If an increase in toxicity occurred as a consequence of translocation by the plant, it seemed probable that the filtrate would be capable of greater dilution without loss of acaricidal activity than a 1-to-800 dilution prepared from the technical octamethyl pyrophosphoramide. As a matter of fact this was not the case. Complete kill of mites was obtained when infested cotton plants were dipped in the plant filtrate at the assumed concentration of 1 to 800. No kill occurred in plants dipped in a comparable filtrate prepared from untreated plants. Complete kill also was obtained when the filtrate was diluted to an assumed concentration of 1 to 8,000. However, at a 1-to-80,000 dilution the plant filtrate gave only 20 per cent kill. On the other hand, a 1-to-80,000 dilution prepared from the compound itself gave a 100 per cent kill, and a 10 per cent kill was obtained with a dilution of 1 to 800,000. In other words, only about one-tenth of the toxicant placed in the nutrient solution could be accounted for in the plant filtrate; hence there was no evidence of an increase in toxicity of the compound due to its translocation by the plant.

SUMMARY.—Octamethyl pyrophosphoramide when taken up by cotton plants, rendered the plants toxic to the cotton aphid, *Aphis gossypii* (Glov.), and to a spider mite. Plants absorbed the compound from the soil through the roots or from sprays applied to the foliage. Dosages of 1 pound of the toxicant per acre were effective when sprays were applied. From 4 to 8 pounds per acre was needed in the soil treatments. Soil treatments made at the rate of 25 pounds per acre was effective for 7.5 months after treatment.

The compound was also highly effective against mites and aphids affecting seedling cotton when it was applied to cottonseed at the time of planting. This method required the lowest application rate of all—from 0.2 to 0.05 pound per acre.

Octamethyl pyrophosphoramide appeared to be highly specific for aphids and mites. It did not kill the boll weevil either as adults or as larvae developing inside squares of treated plants. It was ineffective against the bollworm, the cotton leafworm, the salt-marsh caterpillar the garden webworm, the differential grasshopper, the leafhopper, and the whitefly. Dosages of 16 pounds per acre or more slowly killed adults of the cotton fleahopper; lower dosages were ineffective against this species. After treatment by octamethyl pyrophosphoramide cotton plants did not give off vapors that killed by fumigant action, as has been reported of certain of the phosphorus compounds. The techniques used showed no increase in insecticidal activity of the compound after its translocation by the cotton plant.

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Toxicity of Organic Phosphates to the Two-Spotted Spider Mite and the Foxglove Aphid

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Experiments have been conducted in greenhouses to test the toxicity of aerosols to the nonresistant strain of the two-spotted spider mite, *Tetranychus bimaculatus* Harvey, and to the foxglove aphid, *Myzus convolvuli* (Kltb.). The speed of killing action and the duration of toxicity of aerosols containing 10 per cent of hexaethyl tetraphosphate in methyl chloride have been reported (Fulton *et al.* 1949).

The present paper reports further greenhouse studies on the toxicity of aerosols. These aerosols contain hexaethyl tetraphosphate (12.3 per cent tetraethyl pyrophosphate), tetraethyl pyrophosphate (38.5 per cent), tetraethyl dithiopyrophosphate (95 per cent), tetraethyl monothiopyrophosphate, and tetraisopropyl pyrophosphate. Preliminary experiments with technical octamethyl pyrophosphoramide in aerosols or in aqueous solutions applied to the foliage or in the soil are also reported herein.

PREVIOUS STUDIES.—The compounds studied are derivatives of pyrophosphoric acid and were first prepared by Schrader (1947), who had experimented with them sufficiently to determine that they had insecticidal properties. He found that octamethyl pyrophosphoramide differs from the other compounds discussed in this paper in that it is absorbed by the plant tissue and acts as a systemic pesticide. Ripper *et al.* (1949), in the most extensive report to date on this material, stated that it can enter the plant through the stomata, leaf cuticle, roots, and cut stems. The chemical travels through the plant and is translocated to the growing point. Ripper tested the material against pests on a number of vegetable crops, cotton, pear, apple, and chrysanthemum, and found it to be toxic to 11 species of aphids, four species of mites including *Tetranychus bimaculatus* Harvey, the citrus mealybug *Pseudococcus citri* (Risso), the whitefly *Aleurodes proletella* (L.), and two leafhoppers *Typhlocyba* spp. and *Empoasca lybica* (Bergeuin). Sprays applied at the rate of 3 pounds of the material in 150 gallons of water per acre gave protection of some crops for 6 to 7 weeks. A single application of octamethyl pyrophosphoramide at this dosage was equal to three applications of parathion or hexaethyl tetraphosphate in protecting hops from aphid attack. Plants sprayed with the pyrophosphoramide were more vigorous and yielded better than untreated plants. Ripper *et al.* also stated that the pyrophosphoramide is translocated more efficiently in young growing plants than in older ones.

Bennett (1949) pointed out that toxicity is greatest in leaves, intermediate in shoots, and least in stems. Bennett observed that caged leaves on a stem were non-toxic, whereas those above and below on the same stem were toxic to the same insects.

David & Kilby (1949) stated that, on broad bean, octamethyl pyrophosphoramide in solution was absorbed by the roots and translocated, but that it was not translocated from one leaf to another.

MATERIALS AND METHODS.—The hexaethyl tetraphosphate and tetraethyl pyrophosphate used in the present experiment were from the same commercial sources as those used in previous experiments for the control of greenhouse pests (Smith *et al.* 1948). Tetraethyl dithiopyrophosphate, tetraisopropyl pyrophosphate, and octamethyl pyrophosphoramide were procured in several small lots from the manufacturer, who had made them in laboratory batches or in semicommercial lots. The tetraethyl monothiopyrophosphate was prepared by one of the authors. All these materials were soluble in methyl chloride at the concentrations indicated, and aerosols were prepared without other ingredients.

Weighed quantities of the aerosols, to give the desired dosage for the greenhouse to be treated, were transferred to appropriate cylinders and discharged at temperatures between 75° and 80° F. The fumigation period, during which time the ventilators were left closed, was 2 hours or overnight, except where indicated in studies on residual toxicity. The foxglove aphids were reared on potato, the

two-spotted spider mites on bean, and the other species on the hosts being tested.

The octamethyl pyrophosphoramide for sprays or soil applications was diluted with water 1:500 on a weight basis, or approximately 1:600 by volume. For soil applications the stock solution was made up to 60 ml. for treating plants in 4-inch pots containing 500 ml. of soil and proportionately larger amounts for 5-, 6-, or 7-inch pots containing 900, 1,500, or 2,400 ml. of soil, respectively. Plants in the larger pots were treated with proportionate amounts of the solution, diluted further with water according to the volume of soil. The potting soil was a mixture of composted Evesboro loamy sand and Chester loam with a specific gravity of 1 when air dried.

EXPERIMENTS WITH AEROSOLS.—Studies were made with low dosages of hexaethyl tetraphosphate, tetraethyl pyrophosphate, and tetraethyl dithiopyrophosphate in overnight exposures to determine their relative toxicities to spider mites and certain insects against which they were effective at recommended higher dosages. It will be seen from table 1 that two-spotted spider mites were more susceptible to all three materials than were foxglove aphids. At these low dosages the greenhouse whitefly, *Trialeurodes vaporariorum* (Westw.) and the citrus mealybug, *Pseudococcus citri* (Risso), were not affected. At a dosage of 0.5 gram per 1,000 cubic feet the toxicity to aphids and spider mites of tetraisopropyl pyrophosphate is equal to that of tetraethyl dithiopyrophosphate.

The tests with low dosages of tetraethyl monothiopyrophosphate indicated a high toxicity to aphids and the two-spotted spider mite equal to that of tetraethyl dithiopyrophosphate.

Experiments were made to determine the speed of killing action against spider mites and foxglove aphids by tetraethyl pyrophosphate and against mites by tetraethyl dithiopyrophosphate, and also the duration of effectiveness of the aerosols. Infested plants were placed in the greenhouse and exposed for definite periods after release of the aerosols. At various intervals the plants were replaced.

The data in table 2 show that immediately after release of the tetraethyl pyrophosphate approximately one-third of the mites were killed by a 2-minute exposure and that maximum kills of aphids and mites were obtained within the first 15 minutes of exposure. Toxicity to aphids was less after 30 minutes and declined rapidly after the first hour. The toxicity to spider mites continued for a longer period, but declined rapidly after the second hour. In these tests the speed of killing action and persistence of tetraethyl pyrophosphate were similar to those obtained with hexaethyl tetraphosphate (Fulton *et al.* 1949).

The results with tetraethyl dithiopyrophosphate tested only on spider mites indicated a higher speed of killing action than the tetraethyl pyrophosphate. The dithiopyrophosphate also remained toxic for a slightly longer time in the unventilated greenhouse, but in this respect it more closely resembled the tetraethyl pyrophosphates than it did parathion which remained toxic for several days (Smith *et al.* 1948).

The speed of killing action of tetraisopropyl pyrophosphate released at the rate of 0.5 gram per 1,000 cubic feet of space was not determined. However, its residual toxicity appears to be short since no kill of the potato aphid, *Macrosiphum solanifolii* (Ashm.), was obtained among plants exposed between the 16th and 24th hour after release of the aerosol in an unventilated greenhouse.

An experiment was made with an aerosol containing 5 per cent of octamethyl pyrophosphoramide in methyl chloride which was applied to two groups of actively growing beans and rose plants infested a few hours previously with adults and larvae of resistant and nonresistant spider mites, respectively. The mites were not visibly affected during the first 24-hour period after the release of the aerosol. On the second day, however, a high mortality of mites had occurred, and on the third all mites were dead. The same groups of treated plants were reinfested with nonresistant and resistant mites on the third, ninth, and eleventh days. On the second day after each transfer the mites were dead. The results of the experiment are summarized in table 3.

On older plants with established infestations of mites and with visible feeding injury the adults and larvae were killed on the more succulent leaves in 2 to 4 days, as in the preceding tests. Mites hatching from the eggs or emerging from the quiescent stages were also killed within

Table 1.—Per cent mortality of two-spotted spider mites, aphids, mealybugs, and whiteflies from low dosages of aerosols containing organic phosphates.

Active Ingredient in Aerosol with Methyl Chloride, Per Cent		Grams per 1000 Cubic Feet	Foxglove Aphids	Spider Mites	White Flies	Citrus Mealybugs
Hexaethyl tetraphosphate	1	0.05	60	62	0	0
	5	do.	41	71	0	0
	10	do.	52	68	0	0
	20	do.	39	72	0	0
Tetraethyl pyrophosphate	1	.025	64	84	0	0
		.05	88	97	0	0
	5		84	96	0	0
Tetraethyl dithiopyrophosphate	1	.01	63	96	0	0
	5	.5	99	98	100	98
Tetraethyl monothiopyrophosphate	1	.005	81	98	—	—
		.01	78	98	0	0
Tetraisopropyl pyrophosphate	1	.5	100	99	—	—

1 or 2 days. On the older foliage or on mite-injured leaves the mites were killed more slowly, and a few live mites were present at the end of 14 days. When the treatment was not repeated, these survivors gradually renewed the infestation after another 3 or 4 weeks.

Aerosols containing 5 per cent of octamethyl pyrophosphoramide were also applied to roses in three commercial greenhouses beginning in June. In these ranges the plants had been recently cut back. They were almost devoid of hard old foliage and mites were concentrating on the young new foliage. Two applications of aerosol at 3-week intervals in one greenhouse infested with nonresistant mites resulted in a virtual cleanup, so that no further treatments were required during the subsequent 4-month period. In the other two greenhouses infested with resistant mites, single aerosol applications in June reduced the infestations to low levels. At the beginning of August further treatments were required and four applications were made at 3-week intervals. The mites were destroyed on the young growth, and only an occasional survivor on overlapping older leaves was found throughout the large houses. The infestations did not increase in those houses during the subsequent 2 months.

Table 2.—Mortality of foxglove aphids and spider mites exposed to aerosols containing 5 per cent of tetraethyl pyrophosphate or tetraethyl dithiopyrophosphate for various periods after release (Dosages tetraethyl pyrophosphate 0.25 and tetraethyl dithiopyrophosphate 0.5 gram per 1000 cu. ft.)

Period of Exposure After Release	Tetraethyl Pyrophosphate				Tetraethyl Dithiopyrophosphate	
	Aphids Examined	Per Cent Killed	Mites Examined	Per Cent Killed	Mites Examined	Per Cent Killed
Minutes						
0– 2	—	—	449	30.3	130	88
0 –5	—	—	439	68.3	162	99
0–10	—	—	452	84.5	106	98
0–15	1008	100	591	98.6	229	100
0–30	1060	100	641	97.8	215	100
30–60	1380	71.8	423	95.0	202	100
Hours						
0–1	2287	100	895	97.3	239	100
1–2	2040	18.7	915	87.2	177	100
2–3	2107	6.2	639	28.8	338	100
3–4	—	—	—	—	268	89
4–6	—	—	—	—	167	90
6–8	—	—	—	—	240	70

Table 3.—Per cent mortality of spider mites exposed to 5 per cent octamethyl pyrophosphoramide aerosol at various periods after release. (Dosage 0.5 gram per 1000 cu. ft.)

Days After Release	Nonresistant Mites		Resistant Mites	
	Adults	Larvae	Adults	Larcae
1	0	0	0	0
2	82	92	72	81
3[1]	100	100	100	100
9[1]	100	100	100	100
11[1]	100	100	100	100
20	100	100	100	100

[1] Plants reinfested on these days; subsequent records indicate mortality of transferred mites.

Table 4.—Per cent mortality of spider mites exposed to 5 per cent octamethyl pyrophosphoramide aerosol for various periods after release. (Dosage 0.5 gram per 1000 cu. ft.)

Period of Exposure After Release	2 Days		20 Days	
	Adults	Larvae	Adults	Larvae
Hours				
	Nonresistant mites			
0–12	100	100	100	100
0–2	95	98	100	97
2–12	20	0	74	12
Untreated check	2	0	28	17
	Resistant mites			
0–12	64	82	100	100
0–2	96	100	100	100
2–12	64	51	47	18
Untreated check	3	0	43	19

Apparently the mites were destroyed as they moved to the young leaves containing sufficient quantities of the chemical to be toxic.

In two other commercial greenhouses where foliage was dense on flowering plants, three applications at 3-week intervals in September and October destroyed the mites on the new growth and all except small colonies on the older foliage of an occasional plant.

Because octamethyl pyrophosphoramide shows little or no immediate fumigating action against spider mites, experiments were conducted to determine the period that the ventilators should be kept closed after release of the aerosol.

The results given in table 4 show that a 2-hour exposure is equal in effectiveness to an overnight exposure. Apparently most of the effective particles had fallen by the end of 2 hours, since there was only partial control on resistant or nonresistant mites exposed after this interval.

Soil Applications.—Several replicated tests of a water solution of octamethyl pyrophosphoramide (1 to 600) were made in the soil between May and October 1949. The solution was tested against 13 species of mites and insects on seven kinds of plants in the greenhouse. The results are summarized in table 5. At the dosages tested the material rendered the foliage and stems toxic to six species of aphids and to both resistant and nonresistant strains of the two-spotted spider mite. Periodical reinfestation of treated plants indicated a residual toxicity of 2 to 5 weeks, the time depending upon the dosage.

As shown in table 5, vigorous spring growth of turnip plants in the first test was more toxic to aphids than was the sluggish fall growth of plants in the second test. No evidence of toxicity was observed against adults or larvae of the Mexican bean beetle, the banded greenhouse thrips, the Mexican mealybug, the greenhouse whitefly, the cyclamen mite, or the broad mite.

Of the plants tested chrysanthemum, potato, and snap bean were injured at the highest dosages, but only slight injury occurred at approximately four times the dosage required to kill susceptible species. The injury consisted of chlorosis and necrosis of margins on old leaves. Foliage of treated plants was otherwise darker green, and the growth was more vigorous, which conditions are in agreement with Ripper (1949).

Foliage Sprays.—The same strength water solution of octamethyl pyrophosphoramide was also applied to foliage of bean, chyrsanthemum, lily, and rose in greenhouse tests against the same pests listed in table 5. Complete kills of aphids and two-spotted spider mites were obtained on the sprayed foliage and on new foliage that was not sprayed but was reinfested after the initial treatment. The plants treated by spraying the foliage and those growing in soil treated at the lowest dosages lost their toxicity in about 2 weeks.

In a small-scale test China asters planted in replicated field plots were sprayed three times at 2-week intervals with the water solution of octamethyl pyrophosphoramide, in an effort to control the six-spotted leafhopper, *Macro-*

Table 5.—Effect of applications of octamethyl pyrophosphoramide to soil on potted plants and on associated pests.

MILLILITERS PER POT	SPECIES PRESENT	EFFECT ON PEST	HOST INJURY
	Bean, Stringless Green Pod (4-inch pot)		
7.5[1]	*Tetranychus bimaculatus* (Harvey)[2]	Dead in 6 days	None
15	*Tetranychus bimaculatus* (Harvey)[2]	Dead in 6 days	None
30	*Tetranychus bimaculatus* (Harvey)[2]	Dead in 6 days	Moderate
	Epilachna varivestris Muls.	None	
	Chrysanthemum var. Mary McArthur (6-inch pot)		
22.5[1]	*Anuraphis helichrysi* (Ktlb.)	Dead in 4 days	None
	Aphis gossypii Glover	Dead in 4 days	
	Rhopalosiphum rufomaculatum (Wilson)	Dead in 4 days	
	Hercinothrips femoralis (Reut.)	None	
	Phenacoccus gossypii T. & C.	None	
	Trialeuroides vaporariorum (Westw.)	None	
90	[3]		Slight
100	[3]		Moderate
	Lily (*Lilium longiflorum*) var. White Queen (6-inch pot)		
22.5[1]	*Aphis gossypii* Glover	Dead in 10 days	None
90	[3]		None
180	[3]		None
	Potato var. Katahdin (6-inch pot)		
22.5[1]	*Aphis gossypii*	Dead in 6 dyas	None
	Myzus convolvulvi (Kltb.)	Dead in 6 days	
	Macrosiphum solanifolii (Ashm.)	Dead in 6 days	
	Hemitarsonemus latus (Banks)	None	
	Trialeurodes vaporariorum	None	
90	[3]		Slight
180	[3]		Severe
	Rose var. Talisman (6-inch pot)		
22.5[1]	*Tetranychus bimaculatus*[2]	Dead in 10 days	None
	Saintpaulia var. White Lady (4-inch pot)		
7.5[1]	*Tarsonemus pallidus* Banks	None	None
30	*Tarsonemus pallidus* Banks	None	None
60	*Tarsonemus pallidus* Banks	None	None
	Turnip var. Purple top (7-inch pot, June test)		
18	*Myzus persicae* (Sulz.)	Dead in 4 days	None
36[1]	*Myzus persicae* (Sulz.)	Dead in 4 days	None
	Turnip var. Purple top (7-inch pot, September test)		
36[1]	*Myzus persicae*	88% kill after 20 days	None
72	*Myzus persicae*	99% kill after 20 days	None

[1] Applications of 1:600 solution comparable on basis of soil volume of various-size pots.
[2] Both resistant and nonresistant strains.
[3] Same species of insects reacted similarly to dosages of 90 and 180 ml. per pot.

steles divisus (Uhl.), the vector of aster yellows virus. No reduction in infection was obtained by the treatments.

In a test conducted in May to determine translocation from treaetd foliage, as reported by Ripper (1949), one or two leaves on each of certain turnip plants among several growing in the same pot were dipped in the solution of octamethyl pyrophosphoramide and arranged to avoid contact with untreated foliage. Green peach aphids died within 2 days on treated leaves and in 4 to 6 days on other leaves of the same plants, whereas those on un-

treated plants in the same pots were unaffected. The same experiment, repeated on less active growth of turnips in October, indicated that the material was more slowly translocated because the aphids were killed only on the treated leaves.

David & Kilby (1949) reported no evidence of translocation in foliage of broad bean. Bennett's (1949) finding that caged leaves of willow were nontoxic, whereas leaves above and below the caged leaves on the same stem were toxic was confirmed by the writers in an experiment on chrysanthemums using the crysathemum aphid *Macrosiphoniella sanborni* (Gill.). Possibly high humidity within the cage reduces transpiration and hence the absorption of the material.

It is evident from reports in literature and from our experiments that the efficiency of this material as a systemic poison may vary with the type of host plant, vigor of growth, and conditions affecting transpiration.

SUMMARY.—Aerosols containing hexaethyl tetraphosphate, tetraethyl pyrophosphate, or tetraethyl dithiopyrophosphate in methyl chloride, applied at low dosages, were more toxic to two-spotted spider mites, Harvey, than to foxglove aphids, and were nontoxic to greenhouse whiteflies, and to citrus mealybugs. At the dosages recommended for commercial greenhouses, they were toxic to all four species.

Tetraisopropyl pyrophosphate and tetraethyl monothiopyrophosphate were essentially equivalent to tetraethyl dithiopyrophosphate in their toxicity to aphids and two-spotted spider mites at comparable dosages.

At dosages used in commercial greenhouses, tetraethyl pyrophosphate in aerosols gave a maximum kill of aphids and two-spotted spider mites during the first 15 minutes after release of the aerosol, but it began to lose its toxicity to aphids at the end of 30 minutes and to the mites after the second hour.

In similar experiments tetraethyl dithiopyrophosphate in aerosols gave a higher speed of killing action against two-spotted spider mites than did tetraethyl pyrophosphate. The dithiopyrophosphate also remained highly toxic for about 4 hours in the unventilated greenhouse.

Aerosols containing 5 per cent of octamethyl pyrophosphoramide applied at 1 pound per 50,000 cubic feet gave slow but complete mortality of spider mites on bean and rose. Mites transferred to treated foliage during the 11-day period after treatment were killed. In commercial greenhouses resistant and nonresistant mites were controlled by two to four applications at 3-week intervals.

Six species of aphids and the two-spotted spider mite were killed on foliage of plants growing in soil that had been treated with water solutions of octamethyl pyrophosphoramide.

No injury occurred on seven species of plants sprayed with octamethyl pyrophosphoramide at dosages required for complete kill of susceptible mites and aphids. However, bean, chrysanthemum, and potato plants were slightly injured at four times the required dosage.

Octamethyl pyrophosphoramide was translocated from turnip leaves dipped in the solution to untreated leaves on the same plant.

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Natural and Induced Variations in Two-Spotted Spider Mite Populations

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During the course of investigations on the control of the two-spotted spider mite, *Tetranychus bimaculatus* Harvey, widely different populations of the species were observed. The differences noted were (a) a marked variation in the general appearance of the individuals, and (b) a wide discrepancy in their reaction to acaricide treatments.

Mites on Roses Differ from Those on Beans and Tomatoes.—The difference was first observed in 1939 when an acaricide applied to infested beans and tomatoes at the Experiment Station gave a high degree of control, but did not give a comparable kill when applied to roses in a commercial greenhouse. It had been known for some time that mites on roses are more difficult to kill than are those on a number of other plants. Compton & Kearns (1937) suspected the texture of the rose leaf to be in part responsible for the difference. However, when they took equal numbers of mites from roses and snapdragons and transferred them to cotton cloth and sprayed them with a selenium compound[2], it was found that 100 per cent of the mites from snapdragon were killed and only 2 per cent of those taken from roses.

In the instance observed in Ohio in 1939 the difference was so striking that a comparative study of the two populations was made. It was found that the mites from the commercial greenhouse were distinctly of the two-spotted green type and that no red forms appeared in any stage of development. The mites that were being used in experimental work at the Experiment Station, on the other hand, were predominately red or maroon in color in the adult stage. The color differences were so pronounced that specimens of both lots were submitted to Dr. Philip Garman of the Connecticut Experiment Station and at his suggestion to Dr. E. A. McGregor in California. Both investigators reported that they were unable to find any morphological differences whereby the two lots could be differentiated.

Populations of mites from the two sources were transferred on the same day (March 1, 1939) to two lots of potted bean plants of uniform growth. When the infestations were well established, 10 plants were segregated from each lot. Five plants from each lot were sprayed with one acaricide and the remaining five from each lot with another. Three days later a mortality record was taken by counting the living and dead mites on two leaves taken at random from each plant. The results are given in table 1.

Table 1.—Post-spraying mortality of the two color-phases of the two-spotted spider mite. March 7, 1939.

Acaricide No.	Color Type	Per Cent Mortality: Plant Number 1	2	3	4	5	Mean
1	Red	82	99	90	100	100	94.2
1	Two-spotted green	41	45	50	46	66	49.6
2	Red	100	78	92	81	91	88.4
2	Two-spotted green	44	59	59	53	71	57.2

The data presented in table 1 show that the mites of the two-spotted green type were much more resistant to the action of both acaricides than were the mites of the red color type. An analysis of variance showed that this difference was highly significant at the 1 per cent level.

During succeeding weeks the two populations of mites were maintained separately in the entomology greenhouse and were permitted to feed continuously on beans. In a test made on March 15 they again showed significant differences in the relative numbers surviving acaricidal applications, but the differences were much less than those shown in table 1.

On March 21, 3 weeks after the transfer was made, another acaricide test was carried out. At that time the progeny from the mites feeding on roses were probably in the second or third generation. Three different materials were applied to each

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[2] "*Selocide*."

population in a replicated test and mortality records were taken the next day. The data are shown in table 2.

Table 2.—Post-spraying mortality of the two color-phases of the two-spotted spider mite. March 21, 1939.

Acaricide No.	Color Type	Per Cent Mortality: Plant Number 1	2	3	4	5	Mean
1	Red	89	93	79	88	82	86.2
1	Two-spotted green	79	96	94	100	96	93.0
2	Red	95	100	89	96	81	92.4
2	Two-spotted green	97	94	92	65	77	85.0
3	Red	100	100	100	100	100	100.0
3	Two-spotted green	92	92	100	99	100	96.6

It is evident in table 2 that the differences between the two populations in their response to acaricides had largely disappeared. An analysis of variance shows that the differences were not significant at the 5 per cent level. Apparently the mite populations were being influenced significantly by the host plants on which they were feeding. Color differences, however, could still be detected although they were much less marked.

During the winter of 1939–40 two lots of roses consisting of 30 plants each were grown in sand cultures containing six levels of selenium. One lot of plants was infested with mites of the red color type taken from beans in the entomology greenhouse, and the other was infested with mites of the two-spotted green type taken from roses in the commercial house. On March 21, 1940, about 2 months after the infestation was established, population counts were made on all plants by examining two leaves taken at random from each plant. A summary of the results is given in table 3.

It may be noted from table 3 that when the selenium content in the solution was 1 part per million there was a 98.4 per cent reduction in the red form and only a 46.5 per cent reduction in the green two-spotted form. At this stage the red form was controlled effectively in all instances in which the selenium content in the solution was as much as 1 part per million. The green two-spotted form, on the other hand, was not controlled effectively at any of the levels of selenium used.

During the latter part of April, however, it was observed that all of the plants in this series were becoming heavily infested with mites. By that time the plants infested with the two-spotted green form were largely defoliated. When the experiment was discontinued many of the plants originally populated with the red form were also partially defoliated. Coincident with this finding it was observed that all of the mites were almost entirely green in color. It appeared, therefore, that after feeding for 4 or 5 months on roses the mites of the red form had become more resistant to selenium and had undergone a color change.

Table 3.—Comparative mortality of two color types of the two-spotted spider mite on roses grown in a nutrient solution which contained varying levels of selenium.

Selenium Solution p.p.m.	Mites Per Leaf Five Replicates: Red Type	Green Two-spotted Type	Per Cent Reduction from Check: Red Type	Green Two-spotted Type
0—Check	115	198		
0.5	51	168	55.7	16.2
1	2	106	98.4	46.5
2	0	51	100.0	74.2
3	1	25	99.1	87.4
4	3	23	97.6	88.4

Neiswander & Morris (1940), after getting phenomenal control of the two-spotted spider mite on roses grown in a nutrient solution containing selenium, reported that "Late in the spring a severe infestation of red spiders developed on the plants which had received the higher levels of selenium." The authors were "Unable to explain the change in behavior other than to suggest that the selenium concentration in the foliage may have decreased as the plants became older or that the later generations of mites may have become resistant to selenium at the concentration that was present." It appears now, however, that the red form of the two-spotted spider mites, which was used by Neiswander & Morris in their tests with selenium in nutrient solutions, after feeding on roses for a number of generations became more resistant and consequently were not permanently controlled by the levels of selenium used. In the test described in the current paper it may be noted that the green form of the mite never was controlled satisfactorily

by selenium at the different levels used and that after a number of generations of feeding on roses the response of the red form became similar to that of the green form. It appears, therefore, that a part of the difference in susceptibility can be attributed to the food. It is probable, however, that some specific resistance to selenium was built up in both forms of two-spotted spider mites since early in the course of the experiment there seemed to be some control of the green form by the higher levels of selenium (Table 3).

MITES ON BEAN DIFFER FROM THOSE ON TOMATO.—During succeeding years many laboratory acaricide tests were carried on in the entomology greenhouse in an attempt to develop more effective control measures. The mite population needed for this work was maintained primarily on bean plants growing in pots although both bean and tomato plants were employed in tests. In the preparation of test plants, mites were transferred indiscriminately from old, heavily infested plants to young and vigorously growing plants of either bean or tomato. However, a longer period of time is usually required to develop a suitable population on tomato than on bean. For that reason two or more generations of mites were usually permitted to develop before the tomato plants were used in acaricide tests.

After a considerable number of tests were completed the observation was made that, although the differences were not great, a slightly higher mortality was usually recorded on tomato than on bean following treatment with a given material.

On February 17, 1945 an experiment was set up to test the validity of this observation. Six acaricides were used and each was applied on five bean and five tomato plants. Counts of living and dead mites on each plant were made 3 days later. The average mortality on the five plants that received a given treatment is shown in table 4. It will be observed that each material induced a slightly higher mortality on tomato than on bean.

During 1946 nine acaricides were used at the same strength in one or more replicated tests on both beans and tomatoes (Rodriguez 1946). Although these tests were carried on at different times during the year, the results were tabulated and the data obtained are summarized in table 5. It will be observed that in 35 tests on beans and 20 on tomatoes a consistently higher mortality was again recorded on tomato than on bean. In this series of experiments, therefore, two-spotted spider mites feeding on beans were somewhat more resistant to the action of acaricides than were those feeding on tomato.

Table 4.—Post-spraying mortality two-spotted spider mites on beans and tomatoes sprayed from the same tank—1945.

Acaricide No.	Per Cent Mortality	
	On Beans	On Tomatoes
1	98.6	100.0
2	97.0	98.0
3	82.5	100.0
4	96.6	99.2
5	10.0	20.0
6	7.0	15.0
Mean	65.3	72.0

Table 5.—Post-spraying mortality of two-spotted spider mites on beans compared with that on tomatoes—1946.

Acaricide No.	Host—Beans		Host—Tomato	
	No. Tests	Average % Dead	No. Tests	Average % Dead
1	3	89.8	1	97.6
2	1	24.9	1	68.1
3	5	85.2	4	95.3
4	1	56.3	1	88.6
5	1	65.6	1	93.1
6	8	94.2	3	99.3
7	3	78.6	3	98.0
8	5	82.4	3	98.2
9	8	93.6	3	95.7
Checks	10	5.2	4	6.3
Average		67.6		84.0

MITES RESISTANT TO THE ACTION OF ACARICIDES.—Later in 1946 an experiment was set up in a deliberate attempt to build up resistance to acaricides in two-spotted spider mites (Rodriguez 1946[1]). A mite population on beans was sprayed with a commercial rotenone preparation at a more dilute strength than usual in order to insure that a significant portion of the population would survive. The plants were then placed in a separate room of the greenhouse and the surviving mites transferred to young, vigorously growing,

[1] Unpublished thesis.

bean plants that were not infested. When the population again became sufficiently large to yield reliable mortality records the plants were again sprayed with the same material at the same rate. This process was repeated until the population had been subjected to eight applications of the dilute acaricide.

A mortality record was taken on each of 10 plants 48 hours after the acaricides were applied. Occasionally bean plants taken from the main rearing room of the greenhouse which contained mites that had not previously been subjected to acaricidal treatments, were sprayed in the same manner, at the same time, and with the same material to provide comparisons. The data obtained are summarized in table 6.

Table 6.—Two-spotted spider mite mortality after repeated applications of dilute acaricides.

Date	Population Subjected to Repeated Applications		Check Population for Comparison	
	Application No.	Per Cent Mortality	Application No.	Per Cent Mortality
Acaricide used—a rotenone preparation				
June 19	1	65.0	1	66.1
July 24	2	73.9	1	74.4
30	3	50.3		
Aug. 7	4	43.3	1	85.0
14	5	47.7		
19	6	37.6	1	84.0
29	7	51.7		
Sept. 19	8	52.7	1	86.9
Acaricide used—a dilute dinitro compound				
Aug. 9	1	72.0	1	76.4
19	2	73.0		
Sept. 3	3	65.9	1	76.3
19	4	42.1	1	74.1

After this work was well under way another mite population was isolated and sprayed with a dilute dinitro compound. During a period of 6 weeks this population was subjected to 4 applications in the manner described above. The data obtained with this material are also presented in table 6.

The data show rather definitely that partial resistance to the acaricides was built up in each case during a period of 6 or 7 weeks. The mortality recorded on mites that had not previously been subjected to the action of acaricides remained rather consistent in both cases, whereas that among the mites subjected to repeated applications dropped significantly.

CONCLUSIONS.—Rather wide variations exist among various populations of two-spotted spider mites. The rate at which a population develops, is well as the ease with which it may be destroyed with acaricides, is influenced by the host plants on which the mites feed.

A mite population feeding on roses is usually more resistant to the action of acaricides than one on beans. Likewise a population on beans is more resistant than one on tomatoes. When transferred from one host to another, certain characteristic changes occur during the period in which 2 or 3 generations develop.

A population subjected to the action of a dilute acaricide for three or more generations may develop a partial immunity to that material. It appears that such a heterogeneous organism as the two-spotted spider mite may require that acaricidal materials be changed at frequent intervals in order to insure effective control. A study of the influence of host plant composition on susceptibility or resistance of these forms to acaricides as well as a genetic study of the species should yield valuable information.

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Timing Early Sprays for Summer Control of European Red Mite

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Studies were made for 3 years on the general habits and seasonal development of the European red mite *Paratetranychus pilosus*. The primary objectives were to determine (1) conditions which may lead to serious outbreaks and how to recognize these conditions, (2) the practical relationship, if any, between the brood development and habits of the mite and the regular spray program, and (3) the value of an acaricide in the petal-fall and other early orchard sprays.

Methods.—The relationship of the abundance of winter eggs on the trees to the bronzing of the foilage the following season was studied. Observations were made and mites and eggs were counted in the early season to determine the brood development, the predominant stages of mites present, and the location and movement of the mites.

In 1949 three acaricides—dinitro capryl phenyl crotonate 25 per cent,[1] dinitro-o-cyclohexylphenol, dicyclohexylamine salt 20 per cent,[2] and parathion 25 per cent—were tested on apple trees in two series, differing only in the fungicide used.[3] Four two-tree plots were used for each material in each series. Plot 1 received the treatments in the pink and petal-fall applications, Plot 2 in the petal-fall application, Plot 3 in the petal-fall and first cover applications, and Plot 4 in the petal-fall and second cover applications. The trees in the plots were of moderate size and had a fairly heavy infestation of winter eggs. The sprays were applied with a 100-gallon trailer-type sprayer, using a gun with two nozzles, each with a number 5 aperture disc. A pressure of 375 pounds, which delivered approximately 3 gallons of spray per minute, was maintained; and about 1 gallon of spray was used for each foot of top diameter on trees with dense foliage and 3 quarts on trees with less dense foliage.

The results of the experiment were determined from examinations made preceding and at intervals following the treatments. From 25 to 50 leaves, usually 1 from a spur, were collected from each tree. Each leaf was examined under a binocular microscope and the numbers of dead and live mites and of normal- and abnormal-looking eggs were recorded.

Seasonal History of the European Red Mite.—Figure 1 shows some important points in the seasonal history of the European red mite in Virginia. Ten generations of the mite are represented in the figure. In 1948 winter eggs hatched from April 1 to 26, the peak in hatching coming April 8 to 15. In 1949 they hatched from April 4 to May 4, the peak coming April 18 to 25. The first hatching each year took place about the time apple buds began showing pink and the last at petal-fall. The first mites to hatch had reached maturity and, in some cases, a few second-brood eggs had been deposited by the time the last eggs had hatched.

Counts of 3162 first and second-brood mites and 7390 second-brood eggs in 1948 showed that 75 per cent of the mites and 68 percent of the eggs were on the lower surface of the leaves. Counts in 1947 and 1949 also showed that mites and eggs occurred mainly on the lower surface of the leaves during the first and second broods. Beginning with the third brood, early to mid-June, however, as many mites and eggs occurred on the upper as on the lower surface. Mites from winter eggs were found mainly on the lower surface of the first one or two leaves on a spur, where they remained until approaching maturity and then tended to spread to the upper surface and to new leaves.

These observations indicate that there is a period at petal-fall of apple when the following conditions exist: (1) All winter eggs hatched; (2) very few or no second-brood eggs laid; (3) the apple foliage not mature and with little pubescence on lower side; (4) the mites located mostly on the basal portion of the under side of the first one or two leaves on spurs. All of these conditions, it appears, would favor an efficient mite treatment at petal-fall.

[1] *Arathane*, Rohm and Haas.
[2] *Dn-111*, Dow.
[3] *Fermate*. 76 per cent *Ferbam*, duPont, in series 1; *Crag Fruit Fungicide*, 50 per cent glyoxalidine acetate, 50 per cent isopropanol, *Union Carbide* and *Carbon Corp.*, in series 2.

The time of the season at which mite outbreaks sufficiently serious to cause bronzing of the apple foliage occurred had a definite relationship to the abundance of eggs carried over the preceding winter. If winter eggs are sufficiently numerous to show visibly red around the spurs and buds, an outbreak in late May and early June, resulting in bronzing of the foliage, is almost certain to occur, if not checked by a dormant or delayed-dormant spray (Fig. 1, curve A). A moderate number of winter eggs generally leads to an outbreak with bronzing in July or August (curve B); and a light carry-over of eggs to an outbreak in late summer or fall (curve C), which may reach the bronzing stage.

When bronzing of the foliage occurred in the early season, the injured trees did not recover during the summer, and the size, quality, color, and yield of fruit, as well as bud development for the next year, were affected. Following such an outbreak, the mites almost completely disappeared and did not build up again during the season, with the result that no winter eggs, or more traces of them, could be found on the trees during the following winter. The number of winter eggs resulting from a midsummer outbreak was small to moderate, but a large carry-over was generally the result of late-season outbreaks.

CONTROL EXPERIMENTS.—The results of the experiment are shown in table 1. The data for the two series, in which different fungicides were used, are shown separately.

Parathion ranked first in efficiency. The mites and eggs were so completely destroyed that little build-up was indicated at any time except in the plot receiving only the petal-fall application in series 1. In this plot the number of mites and of eggs found on July 7, the date of the final examination, showed a small but definite increase over that of June 5, indicating that the mites were beginning to build up at that time.

Dinitro capryl phenyl crotonate 25 per cent was decidedly less effective than parathion. Counts showed a satisfactory control for about 10 days following the application of the spray, but the mites generally began to build up after that time. In series 1, the number of eggs was more than 5 times as great on July 7 as on June 5 in the plot receiving only the petal-fall application and more than 4 times as great in the plot receiving the pink and petal-fall applications; while in series 2 the increases were approximately 10 times and 5 times, respectively, in the corresponding plots.

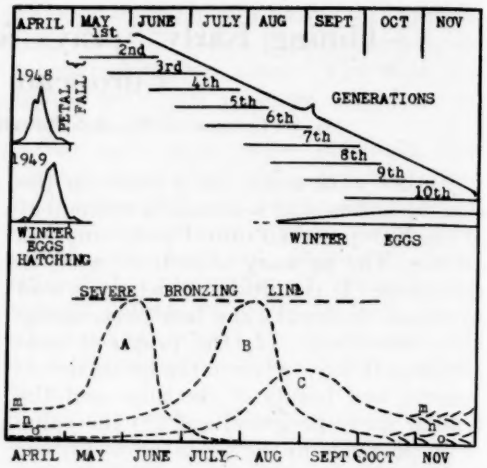

FIG. 1.—Seasonal history of the European red mite. The upper portion shows the time of hatching of winter eggs and the number of generations of the mite. In the lower portion, curve A shows an early-season outbreak resulting from a large carry-over of winter eggs when not controlled by spray; curve B, a midseason outbreak from a moderate carry-over of winter eggs, or from a large carry-over with a poor dormant oil spray job done; and curve C, a late season outbreak from a small carry-over of eggs naturally or from a larger carry-over reduced by a good dormant oil spray job.

Dinitro-o-cyclohexylphenol, a 20 per cent, dicyclohexylamine salt was slower in its killing action than either of the other acaricides tested; but at the end of 10 days following the application, the results were very satisfactory, the number of live mites and eggs being about the same as for dinitro capryl phenyl crotonate 25 per cent. Here also the mites showed a tendency to begin building up again after that time. The final examination, made July 7, showed a definite increase in the mites and eggs present in most plots.

In these experiments an acaricide used in the petal-fall spray on apple gave good control of the European red mite during the period when serious injury by the mite often results following a large carry-over of winter eggs. The addition of a pink, a first cover or a second cover spray did not add much to the control obtained by the petal-fall spray alone during this period. The addition of a cover spray, however, cut down on the amount of build-up indicated by mite and egg

Table 1.—Average number of European red mites and eggs per leaf in the different plots on the dates indicated, 1949.

MATERIALS	TIME OF APPLICATION[1]	May 4		May 12		May 24		June 5		July 7	
		Mites	Eggs	Mites	Eggs	Mites	Eggs	Mites	Eggs	Mites	Eggs
Series 1[2]											
Dinitro capryl phenyl crotonate 25%,[3] 16 oz. to 100 gals.	Pink, petal-fall	.04	.08	.06	.03	0	.22	0	.04	.14	0.18
	Petal-fall	.12	.08	.22	1.6	.62	1.60	.14	.68	.54	3.6
	Petal-fall, first cover	0	.08	.01	0.32	.12	1.4	.04	2.8	.06	0.5
	Petal-fall, second cover	0	.04	.22	3.60	.64	2.7	.18	0.12	.06	0.5
Dinitro-o-cyclohexylphenol, dicyclohexylamine salt 20%,[4] 20 oz. to 100 gals.	Pink, petal-fall	—	0	—	—	.05	.38	0	.2	.08	.8
	Petal-fall	—	.5	.4	5.2	.12	1.5	.04	1.2	.2	2.70
	Petal-fall, first cover	—	.1	.34	3.9	.06	4.0	.02	1.3	.16	1.60
	Petal-fall, second cover	—	0	.02	0.6	.14	1.6	.02	.04	.2	1.70
Parathion 25%,[5] 8 oz. to 100 gals.	Pink, petal-fall	.08	.08	0	.03	0	.06	0	0	0	.12
	Petal-fall	.04	.08	0	.01	.04	.1	0	0	.02	.36
	Petal-fall, first cover	.08	.04	0	0	0	.28	0	.04	.04	.26
	Petal-fall, second cover	0	.04	0	0	.06	.1	0	.12	0	.02
Check (untreated)		2.6	2.6	—	20.0	5.3	22.4	1.7	4.3	—	.02
Series 2[6]											
Dinitro capryl phenyl crotonate 25%,[3] 16 oz. to 100 gals.	Pink, petal-fall	.16	.11	.5	2.8	.24	1.1	.44	1.4	.28	6.7
	Petal-fall	.3	0	.32	3.8	.3	2.9	.4	1.3	.24	14.0
	Petal-fall, first cover	.64	.86	1.6	4.5	.3	2.1	.12	1.1	.28	4.0
	Petal-fall, second cover	.28	.17	.5	2.2	1.0	7.6	.64	3.7	0	1.0
Dinitro-o-cyclohexylphenol, dicyclohexylamine salt 20%,[4] 20 oz. to 100 gals.	Pink, petal-fall	.44	.26	.47	4.4	.3	2.4	.12	3.3	.24	9.0
	Petal-fall	.4	.19	.55	7.0	.54	1.7	.78	4.5	.88	14.5
	Petal-fall, first cover	1.4	—	.8	11.0	.46	7.9	.5	4.8	1.1	20.0
	Petal-fall, second cover	.72	.32	.28	3.5	.76	3.1	.84	2.3	.16	2.7
Parathion 25%, 8 oz. to 100 gals.	Pink, petal-fall	0	0	.12	.07	0	.01	.02	.16	.08	.6
	Petal-fall	0	0	.08	.30	.02	.26	.02	.38	.16	1.64
	Petal-fall, first cover	.6	0	0	.10	0	0	0	0	.1	.78
	Petal-fall, second cover	0	0	.003	.05	.09	.06	0	.02	.04	—
Check (untreated)		4.4	—	1.8	18.6	1.5	7.3	1.9	3.8	1.7	29.7

[1] Series 1: pink, April 17; petal-fall, April 29; first cover, May 12; second cover, May 25. Series 2: pink, April 17; petal-fall, May 2; first cover, May 13; second cover, May 28.
[2] *Fermate* fungicide (du Pont), 76 per cent *Ferbam*, used.
[3] *Arathane* (Rohm & Haas).
[4] *DN-111* (Dow).
[5] *Crag Fruit Fungicide* (Carbide & Carbon), 50 per cent glyoxalidine acetate, 50 per cent isopropanol, used.

counts made on July 7. This was true, with each of the acaricides used and more particularly with dinitro-o-cyclohexylphenol, dicyclohexylamine salt 20 per cent. There was a very large late-season build-up of mites with unusually many winter eggs present in both series. The build-up was consistently worse on the treated trees than on untreated, due perhaps to killing off of natural enemies by the acaricides. The mid- and late-season build-up may be expected to vary from season to season. It appeared that dormant or early season sprays may not give protection through the entire season.

SUMMARY.—European red mite eggs began hatching about the time apple buds showed pink, and the last eggs hatched about petal-fall.

A large carry-over of winter eggs led to outbreaks causing serious bronzing of the apple foliage in the early season, a moderate carry-over to outbreaks causing serious bronzing in mid-summer, and a light carry-over to outbreaks often causing bronzing in late fall. The early-season outbreak was the most serious from the standpoint of injury caused but was followed by mere traces of winter eggs the following season. The last type was least serious but gave a heavy carry-over of winter eggs.

Good control of the early-season outbreak of mites was obtained with a single application of an acaricide, made at petal-fall. An additional application made in the pink, in the first cover, or in the second cover spray did not add much to the control obtained during this time. The second cover spray ranked next in efficiency to the petal-fall spray, and the pink spray was the least efficient. The results of this study indicate that the petal-fall spray is a good alternative for the dormant or the delayed-dormant oil treatment.

Three acaricides—dinitro capryl phenyl crotonate 25 per cent, dinitro-o-cyclohexylphenol, dicyclohexylamine salt 20 per cent, and parathion 25 per cent—were tested. Each gave satisfactory control, parathion proving superior to either of the dinitro preparations used in the experiments discussed.

Insecticidal Control of Aphids and Other Insects on Shade-Grown Tobacco

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Aphids, mainly *Myzus persicae* (Sulz.), have attacked shade-grown tobacco plantings in the Florida-Georgia producing area for four consecutive seasons, 1946 to 1949. During this period they have seriously menaced the crop under a considerable range of climatic conditions. Control experiments were carried out at Quincy, Florida, during the 1949 growing season to advance the limited information made available from 1947 to 1949 on this new control problem. The two main objectives of these experiments were to determine the strength of parathion dust needed for aphid control, and to obtain preliminary data on the value of parathion-DDT dust mixtures for the control of the other major insect pests of shade-grown tobacco.

The dust mixtures tested contained 0.5, 1.0, and 2.0 per cent of parathion plus 10 per cent of DDT. They were prepared from a 15-per cent parathion wettable powder and a 50-per cent DDT wettable powder. The diluent used in each mixture consisted of a finely-ground, non-alkaline, sterilized tobacco dust from which most of the water solubles had been removed.

The 1-acre shade field was divided into three blocks of plots separated by roadways. The plots were 7 rows wide and 38 feet long, and were 8 feet apart. Treatments were arranged in randomized blocks.

RG cigar-type tobacco plants were set in the field on March 15. Twenty-five of these transplants on each plot, five set 2 feet apart on each of the five center rows, came from a plant bed heavily infested with the green peach aphid. The remaining 200 plants on each plot were pulled from a treated bed believed to be free of aphids. The first series of insecticidal treatments was made on March 17, and the schedule was continued thereafter throughout the growing period. All test materials were applied in dust form by means of rotary hand dusters. Applications were made between 7 and 9 A.M. at approximately 8 pounds per acre per application.

The conditions of this control test were more difficult than would normally be encountered under commercial growing conditions because untreated check plots constituted a continuous source of reinfestation throughout the greater part of the growing period. Under commercial conditions small infested areas are usually given spot treatments as soon as discovered. The elimination of these isolated infestations has been found to be an important factor in aphid control and usually reduces the number of allover treatments needed. A survey of tobacco shade fields in this area this season indicated that applications of 1-per cent-parathion dust at about 10-day intervals, supplemented with occasional spot treatments, nearly always gave satisfactory control of aphids.

The total aphid counts for the season indicated that weekly applications of the dust containing 0.5 per cent of parathion plus 10 per cent of DDT exerted significantly less control than weekly applications of the dust containing 1 per cent of parathion plus DDT. No significant differences were apparent when the three tested strengths of parathion plus DDT were applied twice a week. These results and growers' experiences indicate that parathion dust for aphid control on shade-grown tobacco should have a strength of at least 1 per cent when applications are made at weekly or at longer intervals.

Soon after the tobacco transplants had become established, they were attacked by cutworms, consisting mainly of the granulate cutworm, *Feltia subterranea* (F.). Feeding injury became apparent throughout the test area, but ceased on all treatment plots before any appreciable damage had resulted to the stand of young plants. The cutworms injured the untreated plants quite severely, causing a loss in stand of about 10 per cent and considerable leaf injury. Many larvae on these plots were dug out by hand, and poisoned baits were applied to bring the infestations under control. Experiments carried out under very similar conditions during 1948

[1] In cooperation with the North Florida Agricultural Experiment Station.

Table 1.—Control of aphids on shade-grown tobacco by dusts comprised of different strengths of parathion plus 10 per cent of DDT in a tobacco base; Quincy, Fla. 1949.

Parathion Per Cent	Applications			Number of Aphids per 25 Leaves						Per Cent of Commercial Damage
	Number	Interval in days	Pounds per Acre	Apr. 13	May 3	May 17	May 31	June 14	June 28	
0.5	15	7	125	0	74	37	98	150	278	4 to 5
1.0	15	7	125	0	16	1	0	7	18	0
2.0	15	7	124	0	0	0	0	0	0	0
0.5	28	3.5	242	0	18	0	0	0	0	0
1.0	28	3.5	242	0	0	0	0	0	0	0
2.0	28	3.5	243	0	0	0	0	0	0	0
1.0	8	14	65	0	43	7	29	137	217	3 to 4
0 (undusted check)	—	—	—	665	6,696	5,046	6,165	1,455	5,896	100

indicated that applications of a 1-per cent-parathion dust and a dust mixture containing 1 per cent of parathion and 10 per cent of DDT exerted relatively good control of cutworms on young shade-grown tobacco, but the combination dust gave significantly better control than the parathion alone.

Observations made throughout the growing season within the experimental shade field and in commercial plantings of shade-grown tobacco receiving applications of parathion plus DDT indicated that tobacco treated with these insecticides on a seasonal basis sustained no injuries from the tobacco flea beetle, *Epitrix hirtipennis* Melsh., and the garden fleahopper, *Halticus bracteatus* (Say). Both species were generally prevalent during the period in the area on the occasional small patches of untreated tobacco and on untreated vegetable plantings. These observations, as well as limited control tests against these two species, indicate that both of these pests can be controlled with applications of parathion plus DDT.

The dust applications of parathion plus DDT exerted some apparent control of small tobacco hornworms, *Protoparce sexta* (Johan.), that were present in limited numbers in the experimental field during the first part of the growing season. As the season advanced and the infestation increased, the control exerted by these materials proved to be insufficient at all frequency schedules. Observations of commercial plantings receiving parathion and DDT treatments point to the general conclusion that these materials will not give control of heavy tobacco hornworm infestations on shade-grown tobacco.

In the foregoing experiments operators handled and applied the insecticidal materials without apparent ill effects. The same insecticides were used on virtually every shade-grown tobacco crop in the Florida-Georgia area in 1949. A survey disclosed only three cases of illness apparently caused by the inhalation of or contact with parathion. In these cases operators of hand dusters suffered slight and temporary illness, which did not require medical attention.

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Six Years Field Tests with Insecticidal Dusts Against Pea Aphid[1]

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During the last six years several new insecticides, including DDT, hexaethyl tetraphosphate, tetraethyl pyrophosphate and parathion, have been tested in Wisconsin against the pea aphid, *Macrosiphum pisi* (Kltb.), a pest of the canning pea crop.

Harries (1944), in laboratory tests with DDT dust mixtures, found that this material was toxic to the pea aphid at concentrations as low as 0.1 to 0.6 per cent. Potts *et al.* (1945) tested DDT in the form of atomized concentrated sprays under field conditions and found it effective against the pea aphid.

Bronson & Hall (1946) showed that hexaethyl tetraphosphate was much superior to nicotine in laboratory tests against the pea aphid.

Creighton & Gresham (1947), in field tests with dust mixtures, found parathion to be highly effective against the green peach aphid. Anderson & Hofmaster (1948) found that a dust mixture containing only 0.25 per cent of parathion was not very toxic to the pea aphid under field conditions.

Dudley *et al.* (1948) in field tests found that DDT, tetraethyl pyrophosphate, and parathion gave a high degree of control of the pea aphid.

In this report only the toxicants that have been tested separately are included. Mixtures of two or more materials such as DDT, rotenone, nicotine, pyrethrum, an aliphatic thiocyanate, piperonyl cyclonene and *n*-Propyl Isome were also tested, but in general the results of these tests were not outstanding.

The insecticides were applied as dusts to approximately 1/6-acre plots of the Perfection variety of peas in replicates of six. Applications were made with a power duster equipped with a 24-foot boom, traveling at the average rate of 2½ miles per hour. A 45-foot apron, or trailer, was employed except when the plants were wet with dew or the wind velocity was not over 4 miles per hour. A positive feeding device (Bronson & Dudley 1944) made it possible to regulate the application to 35 pounds of dust per acre (plus or minus 5 per cent). Only one application was made each year.

One formulation of derris and one of DDT have been tested for the years 1944 to 1949 and are considered as standards of comparison for the newer insecticides. Tests with some of these insecticides were discontinued because of poor performance, and those which showed the greatest promise have been tested at reduced concentrations. In 1944 pyrophyllite was used as the diluent, whereas in the other years talc was the principal diluent for all formulations except parathion, which was diluted with a clay.

When the weather permitted, the aphid infestation was determined 24, 48, and 72 hours after application and thereafter at two-day intervals up to approximately 10 days after treatment. The sampling procedure consisted in walking the length of each plot, making five single sweeps at random with a net 15 inches in diameter. The number of aphids collected was determined for each sweep and the average of the five sweeps then became the unit of infestation.

In presenting the results (Table 1) it appeared advantageous to select, in so far as possible, the six-day period following application because it better reflected the toxicity of insecticides that acted more slowly than others. For 1947, however, the three-day rather than the six-day infestation is given, because the aphid infestation decreased so rapidly after the third day that the effect of the insecticides was obscured. During the first 3 years (1944 to 1946) untreated checks were not provided for the experiments, their purpose being primarily to compare the performance of different materials, but for the last three years it was deemed advantageous to include checks so that the reduction in infestation could be determined for all materials tested. The checks were not used in making analyses for significant differences.

The various formulations used and the results obtained are shown in table 1.

[1] In cooperation with the Wisconsin Agricultural Experiment Station.

Table 1.—Effectiveness against pea aphids of various insecticidal dusts applied on field plots of peas. Madison, Wis., 1944–1949.

DUST MIXTURES (NUMERALS ARE PERCENTAGES OF STRENGTH)	POUNDS TOXICANT PER ACRE	SURVIVING APHIDS PER SWEEP	PER CENT CONTROL
1944 experiment (after 6 days)			
Derris (rotenone 0.75), mineral oil 2	0.3	16	—
DDT, mechanical mix			
5	1.8	25	—
2.5	.9	58	—
Difference required for significance at 5% level	—	22	—
1945 experiment (after 6 days)			
Derris (rotenone 0.75), mineral oil 2	.3	3	—
DDT 5, mechanical mix	1.8	4	—
DDT 5 in volatile solvent	1.8	2	—
Nicotine sulfate-lime (nicotine 2)	.7	38	—
Ryania 40	14	98	—
Difference required for significance at 5% level	—	31	—
1946 experiment (after 7 days)			
Derris (rotenone 0.75), mineral oil 2	.3	43	—
DDT 5, mechanical mix	1.8	10	—
DDT in volatile solvent			
5	1.8	6	—
2.5	.9	9	—
1.25	.45	30	—
Methoxychlor 5	1.8	97	—
Benzene hexachloride, gamma isomer 0.5	.2	129	—
Difference required for significance at 5% level	—	28	—
1947 experiment (after 3 days)			
Derris (rotenone 0.75), mineral oil 2	.3	13	87
DDT 5 mechanical mix	1.8	20	80
DDT in volatile solvent			
2.5	.9	21	78
1.25	.45	26	73
DDT 1.25 in nonvolatile solvent 2.3	.45	12	87
Parathion 0.5	.2	9	91
Tetraethyl pyrophosphate			
5	1.8	6	94
2.5	.9	4	95
Hexaethyl tetraphosphate 5	1.8	38	61
Benzene hexachloride (gamma isomer 0.85)	.3	32	67
Difference required for significance at 5% level	—	15	—
Check, untreated	—	98	—
1948 experiment (after 6 days)			
Derris (rotenone 0.75), mineral oil 2	.3	51	90
DDT 5, mechanical mix	1.8	20	96
DDT 2.5 in volatile solvent	.9	20	96
DDT 1.25 in nonvolatile solvent 2.3	.45	28	95
Parathion			
0.5	.2	24	95
0.25	.1	27	95
Tetraethyl pyrophosphate 1	.4	31	94
TDE 5	1.8	20	96
Difference required for significance at 5% level	—	10	—
Check, untreated	—	516	—
1949 experiment (after 6 days)			
Derris (rotenone 0.75), mineral oil 2	.3	7	93
DDT 5, mechanical mix	1.8	2	98
DDT 1.25 in nonvolatile solvent 2.3	.45	4	96
Parathion			
1	.4	2	98
0.5	.2	2	98
Tetraethyl pyrophosphate 0.75	.3	4	96
Difference required for significance at 5% level	—	2	—
Check, untreated	—	103	—

The volatile solvents were acetone, carbon tetrachloride or trichloroethylene. The nonvolatile solvent was a proprietary mixture consisting chiefly of di- and trimethylnaphthalenes. All three parathion formulations were proprietary mixtures.

Parathion and tetraethyl pyrophosphate are not considered to have any prolonged insecticidal toxicity yet they have compared favorably with DDT and have been superior to rotenone in keeping the aphid infestation at a low level. In 1947, as already explained, there are no records beyond the 3-day period following application. In 1948, however, 13 days after application, and in 1949, 8 days after application, the average infestation in the plots treated with the phosphate formulations was slightly lower than in the DDT-treated plots and considerably lower than in the rotenone-treated plots. It is believed that these results may be explained in part by the very high initial kill effected by the phosphates.

DISCUSSION OF RESULTS.—Over the 6-year period DDT, 5-per cent mechanical mix, was on the average superior to rotenone, 0.75 per cent, and exhibited a greater uniformity in reducing the infestation. DDT, 5 per cent in a volatile solvent, gave the best performance the 2 years it was tested. DDT, 2.5 per cent in a volatile solvent, and 1.25 per cent in a nonvolatile solvent, each tested 3 years, were about as effective as DDT, 5-per cent mechanical mix. DDT, 1.25 per cent in a volatile solvent, tested 2 years, was less effective than the other DDT formulations.

Parathion, 0.5 per cent, tested 3 years, was superior to DDT, 5-per cent mechanical mix and 1.25 per cent in nonvolatile solvent. The two concentrations of parathion—0.5 and 0.25 per cent—were about equally effective in 1948, as were the 1-per cent and 0.5-per cent cent concentrations in 1949.

The concentration of tetraethyl pyrophosphate was reduced from year to year, and in 1949 a concentration of 0.75 per cent provided highly satisfactory aphid control, practically equal to that of the other materials.

TDE was as good as any other material the one year it was tested. Hexaethyl tetraphosphate, nicotine, benzene hexachloride, methoxychlor, and ryania were unsatisfactory at the concentrations employed.

Derris with a rotenone content of 0.75 per cent, when combined with a light mineral oil, gave satisfactory control of the pea aphid four of the 6 years. DDT, 1.25 per cent in a nonvolatile solvent, was almost as effective as higher concentrations of DDT. Parathion, 0.5 per cent, was effective the three years it was tested. Tetraethyl pyrophosphate was effective at concentrations of 1 per cent and 0.75 per cent, although results for each concentration are for 1 year only.

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Factors Influencing Compatibility of Benzene Hexachloride and Parathion with Calcium Arsenates[1]

C. F. Rainwater and E. E. Ivy, *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*[2]

Field-plot experiments with mixtures of benzene hexachloride and calcium arsenate have led to the conclusion that benzene hexachloride is incompatible with ordinary commercial calcium arsenate (Ewing *et al.* 1947, Becnel *et al.* 1947, Rainwater & Bondy 1947, Gaines & Young 1948). Ewing *et al.* (1947) reported that in laboratory tests with the cotton aphid, *Aphis gossypii* Glov., a special lime-free calcium arsenate was apparently compatible with benzene hexachloride. Subsequent field-plot tests with this special calcium arsenate in mixtures with benzene hexachloride indicated that these materials were compatible. Ordinary calcium arsenate was also found to be compatible with benzene hexachloride in the field tests (Ewing & Parencia 1948). Dunnam & Calhoun (1948) also reported that one mixture of calcium arsenate and benzene hexachloride appeared to be compatible, whereas another was apparently incompatible.

It was generally thought that mixtures of parathion and calcium arsenate would be incompatible, since it was known that parathion breaks down when mixed with alkaline diluents. However, laboratory tests conducted at Waco, Texas, and field tests at Tallulah, La., during 1947 (unpublished data) indicated that freshly mixed calcium arsenate-parathion dusts gave good aphid control. Roussel & Gaines (1949) reported satisfactory aphid control with a mixture of parathion and a special calcium arsenate.

Laboratory experiments were conducted at College Station, Texas, to determine the comparative degree to which benzene hexachloride and parathion are compatible with regular commercial calcium arsenate, and the influence of different storage conditions on the effectiveness of the various mixtures. Two special experimental calcium arsenates were included in the tests with mixtures containing benzene hexachloride, and one of the special calcium arsenates was included in the tests with parathion mixtures.

The dust mixtures were formulated to contain benzene hexachloride, 1 per cent of the gamma isomer, or 1 per cent of parathion. The benzene hexachloride used in preparing the mixtures was a dust concentrate containing 6 per cent of the gamma isomer and 44 per cent of other isomers. The parathion was a 15 per cent dust concentrate. Mixtures were made in which eight regular commercial brands of calcium arsenate from eight manufacturers were used, as well as the two experimental calcium arsenates. In addition, mixtures were prepared with pyrophyllite as a diluent.

All mixing was done in a laboratory ball mill. Immediately after being prepared the mixtures were divided into three portions. One portion was placed in a tightly stoppered glass specimen bottle and was held in the laboratory at ordinary room temperature. The temperature range in this room during the period of storage was from 74° to 96° F. Another portion was placed in a paper bag and held in an incubator at 140° F. The relative humidity in the incubator varied between 5 and 15 per cent. A third portion was placed in a paper bag and held in a moist chamber at 100 per cent relative humidity and at room temperature (74° to 96° F.).

The cotton aphid was the test insect used to determine the comparative effectiveness of the various mixtures held under different conditions of storage. All mixtures were applied as dusts to infested cotton leaves in a laboratory dusting tower at a rate of 8.67 pounds per acre. Net mortalities were based on the number of live and dead aphids at the end of a 24-hour exposure. Each test was replicated three to five times, each replicate being conducted on a different day. Tests were conducted 1 week, 5 or 6 weeks, and 20 weeks after the mixtures were prepared.

Most of the dusts containing calcium arsenate were somewhat less effective than the pyrophyllite dusts. Mixtures stored at 140° F. or at 100 per cent rela-

[1] Report of a study made under the Research and Marketing Act of 1946.

[2] In cooperation with the Texas Agricultural Experiment Station.

Table 1.—Per cent loss in effectiveness of parathion and gamma benzene hexachloride in mixtures with different calcium arsenates and pyrophyllite when tested at different intervals after mixing and under different conditions of storage. [The cotton aphid was the test insect.]

	1 PER CENT OF PARATHION AND STORED IN—							1 PER CENT OF GAMMA BHC AND STORED IN—						
	BOTTLES AT 74°–96° F.			PAPER BAGS AT 140° F.		PAPER BAGS AT 100% RELATIVE HUMIDITY		BOTTLES AT 77°–96° F.			PAPER BAGS AT 140° F.		PAPER BAGS AT 100% RELATIVE HUMIDITY	
MIXTURE No. 1	1 Week	5 Weeks	20 Weeks	5 Weeks	20 Weeks	5 Weeks	20 Weeks	1 Week	6 Weeks	20 Weeks	6 Weeks	20 Weeks	6 Weeks	20 Weeks
1	1	46	98	56	99	81	99	0	57	69	27	65	89	88
2	17	43	99	31	92	80	100	11	33	38	44	38	63	78
3	4	55	98	66	86	75	98	6	16	0	40	59	43	49
4	8	14	78	39	99	91	99	0	24	19	35	58	34	63
5	0	8	10	32	93	31	99	0	0	1	29	70	48	69
6	0	11	14	20	69	21	98	0	29	56	51	75	91	86
7	3	0	20	48	98	2	0	0	11	0	9	71	45	34
8	3	0	5	37	80	16	27	0	0	0	49	73	1	42
9	0	0	2	20	55	6	1	0	0	76	17	24	0	35
10	—	—	—	—	—	—	—	0	0	0	17	33	23	26
11	0	0	0	3	5	0	0	0	0	0	31	65	29	31
Difference required for significance, 5 per cent level		15	11	16	12	15	8		17	25	21	12	17	16

[1] Mixtures 1 to 8 were made from different sources of regular commercial calcium arsenate, mixtures 9 and 10 were made from special lime-free calcium arsenates, and mixture 11 was made from pyrophillite.

tive humidity were less affected than mixtures stored at room temperatures in sealed containers.

In order to calculate the loss in effectiveness of parathion and gamma benzene hexachloride in the various mixtures, the pyrophyllite mixture, stored in tightly stoppered glass bottles and held at room temperature, was used as a standard. The calculations were made on the basis of the kill from the various mixtures under test at the same time. Because of variation in susceptibility of aphid populations from time to time, comparisons should not be made with mixtures tested at different times. The data are presented in table 1.

Rather striking differences were found in the degree of compatibility of the various calcium arsenates with benzene hexachloride and with parathion. In general, the calcium arsenates which were most incompatible with parathion were also quite incompatible with benzene hexachloride. The two special calcium arsenates were more compatible than certain of the commercial calcium arsenates, but no more so than other commercial calcium arsenates.

Samples of the calcium arsenates were analyzed by the Division of Insecticide Investigations, Bureau of Entomology and Plant Quarantine. The samples contained between 39.0 and 52.3 per cent of arsenic pentoxide, 0.6 to 17.1 per cent of which was water soluble. No correlation was found, however, between the arsenic pentoxide content and the degree to which the samples were compatible with benzene hexachloride or parathion. The free-lime content of the various calcium arsenates was determined by the Texas Agricultural Experiment Station, but unfortunately the analyses were made 90 days after the calcium arsenate mixtures were prepared, and hence do not show the free-lime content at the time of mixing. It has since been determined that the free-lime content of small samples of calcium arsenate stored in paper bags decreases rather quickly.

Slight losses in effectiveness occurred with certain of the calcium arsenate-parathion mixtures within 1 week after mixing, but these losses were not significant. Five weeks after mixing, three of the mixtures stored in bottles were significantly less effective whereas all but two of the mixtures stored in the moist chamber had undergone significant reduction. All nine of the mixtures stored in the incubator were significantly reduced. Twenty weeks after mixing all but three of the mixtures of calcium arsenate and parathion stored in bottles were less effective, and all but two of those stored in the moist chamber. All nine samples stored in the incubator were greatly affected. With the pyrophyllite-parathion mixtures no significant loss occurred in either the incubator or the moist chamber.

No significant loss in effectiveness occurred in the mixtures of calcium arse-

nate and benzene hexachloride during the first week of storage. Six weeks after mixing four of the mixtures stored in bottles had undergone significant reduction, compared with eight stored in the moist chamber. All calcium arsenate-benzene hexachloride mixtures stored either in the moist chamber or the incubator were significantly less effective after 20 weeks of storage, whereas only four of the mixtures stored in bottles were thus reduced.

In general, the special experimental calcium arsenates were more compatible than the commercial product. For example, a parathion mixture made from sample No. 9, a special calcium arsenate, was significantly more effective than any of the commercial calcium arsenate mixtures after being held in the incubator for 20 weeks. Special calcium arsenates Nos. 9 and 10 were significantly more compatible with benzene hexachloride than seven of the eight commercial calcium arsenates when held in the incubator for 20 weeks.

Of the mixtures held for 20 weeks in the moist chamber, only one commercial calcium arsenate, sample No. 7, was equal in compatibility with parathion to special calcium arsenate No. 9. Samples No. 7 and 8 were the only commercial calcium arsenates which equaled sample No. 9 in compatibility with benzene hexachloride under moist storage conditions. The pyrophyllite-benzene hexachloride mixtures were significantly reduced in effectiveness in the incubator and also in the moist chamber.

EFFECT OF THE DIFFERENT ISOMERS OF BENZENE HEXACHLORIDE ON COMPATIBILITY.—Not only were there differences in the degree of compatibility of benzene hexachloride with different calcium arsenates, but there were also differences in the degree of compatibility of benzene hexachloride from different sources with any given calcium arsenate. Mixtures made from the pure gamma isomer lost their effectiveness much more rapidly and completely than mixtures made from technical benzene hexachloride containing the other isomers. This suggested that the other isomers were in some way interfering with the reaction between the gamma isomer and some component of calcium arsenate.

To clear up this point, mixtures were prepared from pure isomers of benzene hexachloride furnished by the Division of Insecticide Investigations. A sample of calcium arsenate known to be highly incompatible with benzene hexachloride was selected (sample No. 2). When 1 per cent of the pure gamma isomer was mixed with this calcium arsenate, the mixture rapidly became less effective against the cotton aphid. In 2 weeks the loss in effectiveness amounted to 92 per cent.

When mixtures were prepared containing 6 per cent of the beta isomer in addition to 1 per cent of the gamma isomer, the loss in effectiveness during a 2-week period was 86 per cent. Six per cent of the alpha isomer reduced the loss to 66 per cent, and to 65 per cent when 6 per cent of the epsilon isomer was included. Most outstanding, however, was the effect of the delta isomer. No loss occurred in a mixture containing 1 per cent of the gamma isomer with 6 per cent of the delta isomer. None of the isomers other than the gamma isomer showed appreciable toxicity to aphids when tested separately in calcium arsenate mixtures.

Cristol (1947) found that the delta isomer of benzene hexachloride did not react with alkaline substances in the same way as did the alpha and gamma isomers. He suggested that with the delta isomer, the first step, conversion to pentachlorocyclohexene, occurred much more rapidly than with the other two isomers. He also presented evidence to show that with the delta isomer, the rate-determining step in dehydrochlorination was the elimination of the second mole of hydrochloric acid.

An experiment was conducted to determine the effect of various amounts of the delta isomer on mixtures of benzene hexachloride and calcium arsenate containing various proportions of lime. The calcium arsenate used was special calcium arsenate No. 10, and at the time of mixing it contained 0.5 per cent of free lime. Mixtures containing larger amounts of lime were prepared by mixing chemically pure calcium hydroxide with this calcium arsenate. Various amounts of the delta isomer were added to the mixtures, as is shown in table 2. Each of the mixtures contained 1 per cent of the pure gamma isomer of benzene hexachloride.

Aphid toxicity tests conducted immediately after the samples were prepared indicated that there were no significant differences in the effectiveness of any of

Table 2.—Results of laboratory test 2 to determine the degree of protective action afforded by various amounts of the delta isomer of benzene hexachloride to the gamma isomer in the presence of varying degrees of alkalinity. [Mixtures had been held in glass bottles in the laboratory for 3 months.]

1% Pure Gamma Isomer of Benzene Hexachloride in Calcium Arsenate Containing Indicated Per Cent of Free Lime	Average Per Cent Net Kill When the Mixtures Contained the Indicated Per ent of Delta Isomer				Per Cent Loss in Effectiveness When the Mixtures Contained the Indicated Per Cent of Delta Isomer			
	None	1	2	4	None	1	2	4
0.5	36.9	—	—	—	2	—	—	—
4.5	35.9	31.9	37.0	41.4	5	15	2	0
8.5	29.5	33.3	32.9	35.5	22	12	13	6
16.5	3.2	20.1	25.2	42.7	92	47	33	0
Pyrophyllite mixture	37.7	—	—	—	—	—	—	—

the samples. The tests were repeated 3 months later. In this second test, a freshly prepared dust containing 1 per cent of pure gamma benzene hexachloride gave an average kill of 37.7 per cent. As is shown in table 2, several of the mixtures were significantly reduced in effectiveness. The greatest reduction was in mixtures containing the most lime. At the high lime levels the protective action of the delta isomer was again clearly demonstrated.

SUMMARY.—Eight commercial calcium arsenates and two special calcium arsenates were each mixed with benzene hexachloride and with parathion. After various periods of storage the dust mixtures were tested against the cotton aphid, *Aphis gossypii* Glov. Some of the commercial calcium arsenates were extremely incompatible with both benzene hexachloride and parathion. The special calcium arsenates and certain of the commercial calcium arsenates appeared to be more compatible than others; however, no instance of complete compatibility under all storage conditions was found. High temperature and high humidity were each conducive to rapid loss in effectiveness of the mixtures.

In tests conducted with a special calcium arsenate containing various concentrations of free lime, the most complete loss in effectiveness of the gamma isomer of benzene hexachloride occurred in mixtures containing the highest lime concentrations. Mixtures prepared from the pure gamma isomer were less effective than mixtures prepared from the technical benzene hexachloride containing the other isomers. The delta isomer was outstandingly effective in protecting the gamma isomer in mixtures of calcium arsenate and benzene hexachloride.

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Contamination of Meat and Milk by Chlorinated Hydrocarbon Insecticides Used for Livestock Pest Control[1]

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In the development of insecticides for the control of ectoparasites of livestock, their possible toxicity to mammals has been an important consideration. Not only must the material be harmless to the animals, but it must not be absorbed and stored in the edible tissues or excreted in the milk. Research on these problems has been undertaken at Kerrville, Tex., laboratory of the Bureau of Entomology and Plant Quarantine, by entomologists and chemists of this Bureau and a veterinarian of the Bureau of Animal Industry, in cooperation with workers of the Texas Agricultural Experiment Station and, in some experiments, with the insecticide industry. Experiments with the chlorinated hydrocarbon insecticides—DDT, TDE, benzene hexachloride (technical), lindane, toxaphene, chlordane, and methoxychlor—are summarized in this paper. Reports of some of this work have already been published.

Materials and Methods.—The insecticides were applied to cattle, sheep, and goats as sprays or dips made with commercially formulated wettable powders or laboratory-prepared emulsion concentrates composed of 25 per cent of insecticide, 65 per cent of xylene, and 10 per cent of emulsifier.[3]

The animal tissues and milk were analyzed for DDT and TDE by the colorimetric method of Schechter *et al.* (1947). The nonspecific organic-chlorine method described by Carter (1947) was employed in testing for the other insecticides.

Storage of Insecticides in Animal Tissues.—*Experiments with Sheep and Goats.* It is generally believed that the chlorinated hydrocarbons are deposited in the fatty tissues. In the case of certain heavily treated animals, analyses of muscle have given negative results, whereas large amounts of the insecticides have been found in body fat, omental fat, and kidney fat. Claborn & Beckman[4] have found that the storage is about the same in all three types of fat.

Bushland *et al.* (1948) dipped sheep and goats in xylene emulsions or wettable-powder suspensions of the chlorinated hydrocarbon insecticides. The insecticides were used at a concentration of 1.5 per cent, and treatments were repeated at 4-day intervals until eight applications had been made. Those treatments represented great overdosage as far as practical insect control is concerned, but the tests were designed to determine whether such heavy treatments could induce poisoning. The amount of insecticide stored in the fat of treated animals and the duration of such storage were also studied by Claborn, *et al.*[5] One month after the last dipping a sheep from each treatment group was slaughtered, and organic-chlorine determinations were made on fat samples taken at autopsy. Six months later another sheep and a goat from each group were slaughtered and additional fat samples analyzed. The results are assembled in table 1 which shows that, when excessive treatments are applied, animals store relatively large amounts of the insecticides in their fat. It is assumed that the animals slaughtered after each interval were representative of their treatment groups. After 7 months the insecticide levels in the fat had dropped considerably, but all six insecticides were still present in appreciable amounts.

Nineteen months after treatment, fat was taken by biopsy from one or two goats in each group treated with DDT or TDE. Analyses on that fat gave negative results, indicating that those insecticides had disappeared.

Storage of DDT in Beef Fat.—In another experiment Wells, Claborn, Beckman, and Radeleff (unpublished data) sprayed eight Hereford cows with 0.5-per cent emulsions and suspensions containing 0.5 per cent of DDT five times at 28-day

[1] Presented at the meeting of the American Association of Economic Entomologists at Tampa, Fla., December 13–16, 1949 Report of a study made under the Research and Marketing Act of 1946.
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[3] *Triton X-100.*
[4] Claborn & Beckman (unpublished).
[5] Claborn, Beckman & Radeleff (unpublished).

Table 1.—Parts per million of insecticide found in fat of sheep and goats after eight treatments at 4-day intervals with dips containing 1.5 per cent of various chlorinated hydrocarbon insecticides.

Insecticide Formulation	After 1 Month	After 7 Months	
	Sheep	Sheep	Goats
DDT:			
Wettable powder	124	26	29
Emulsion	126	22	28
TDE, emulsion	70	36	30
Methoxychlor, emulsion	95	18	34
Toxaphene:			
Wettable powder	42	10	19
Emulsion	73	34	10
Chlordane:			
Wettable powder	254	19	—
Emulsion	112	25	—
Benzene hexachloride (12 per cent gamma), emulsion	352	32	18

intervals for control of horn flies, *Siphona irritans* (L.). All the cows had calves, but only four calves were treated. The others were left unsprayed so that the effects of ingesting DDT in the milk could be measured. Milk samples taken from the cows periodically during the course of treatment were found to contain DDT. One month after the last treatment all the cows and calves were slaughtered, and fat from each animal was analyzed by the colorimetric method.

The five sprayings resulted in an average storage of 15 p.p.m. (range 8 to 22 p.p.m.) of DDT in the fat of treated cows. The calves that suckled treated cows but were not sprayed had an average of 25 p.p.m. (range 18 to 35 p.p.m.) of insecticide in their fat, whereas calves that were sprayed with DDT and also consumed the insecticide in milk stored 52 p.p.m. (range 36 to 73 p.p.m.) of DDT in their tissues.

These figures are believed to represent about the maximum contamination that would result from horn fly control, as there are very few regions in this country where as many as five sprayings would be required for control of horn flies during one season.

The indications of this experiment were confirmed in a practical study. At the end of the 1949 fly season 18 recently weaned Hereford steers were obtained from three ranches in the vicinity of Kerrville. Analyses of fat samples taken by biopsy showed the DDT content to range from 7.1 to 74.8 and average 32.5 p.p.m.

To calculate the contamination of edible beef, these values should be divided by 12, as market meat is reported by Edelmann *et al.* (1943) to contain about 8 per cent of fat.

STORAGE OF TOXAPHENE, CHLORDANE, AND BENZENE HEXACHLORIDE IN BEEF FAT.—Radeleff and coworkers recently completed experiments wherein Hereford cattle were sprayed 12 times at biweekly intervals with emulsions containing 0.5 per cent of toxaphene, 0.5 per cent of chlordane, or 0.025 per cent of gamma benzene hexachloride (mixed isomers containing 12 per cent of gamma). In one experiment 36 Hereford steers held on a fattening diet were sprayed with a kerosene-base emulsion of toxaphene. Samples of omental fat taken from all the animals by biopsy before treatments were begun averaged 4.8 p.p.m. of organic chlorine. Animals were selected for biopsy sampling after different numbers of sprayings. If these fat samples contained significantly more organic chlorine than those from untreated animals, the animals were withdrawn from treatment and biopsies were made periodically until the chlorine level returned to normal. No significant increases in organic chlorine were noted after one spraying. Increases did occur in steers treated 2, 3, 4, and 6 times, but the levels returned to normal within a few weeks. Animals sampled after 8, 10, or 12 sprayings did not show significant increases in the organic-chlorine content of their fat.

In another experiment groups of Hereford cattle, each consisting of four steers and four heifers, held under range conditions, were sprayed with emulsions containing the different insecticides. Four untreated steers served as checks.

Fat samples taken from cattle sprayed 12 times with a kerosene-base toxaphene emulsion averaged 11 p.p.m. of organic chlorine 2 weeks after the last spraying, but 4 weeks later the organic-chlorine content had returned to normal. In the group treated with a xylene emulsion of toxaphene organic chlorine averaged 8 p.p.m. 2 weeks after the last treatment and did not change during the following 4 weeks. This increase over the 4.8 p.p.m. organic chlorine for the average of the untreated animals was probably not significant.

The eight animals treated with the chlordane emulsion averaged 17 p.p.m. of organic chlorine in their fat 2 weeks after the twelfth treatment. Four weeks later the average had dropped to 6, and 10 weeks after the last spraying it had returned to an apparent normal of 5 p.p.m. The fat of animals treated with benzene hexachloride showed 26 p.p.m. of organic chlorine 2 weeks after the twelfth spraying. Four weeks later the average was 11, and 10 weeks after sprayings were completed the average was 4 p.p.m.

The insecticides were sprayed at a concentration that might be employed in tick control. The 2-week interval represented the maximum frequency of application in practical field use. The number of treatments probably exceeded any that would be normally applied in this country.

In another experiment lindane mixed with feed at dosages of 1, 10, and 100 p.p.m. was fed to Hereford cattle for 12 weeks, two animals receiving each dosage. After 2 weeks' feeding at the highest dosage about 65 p.p.m. of lindane was present in the fat. Fat samples taken in biopsies made after 10 weeks' feeding contained, respectively, about 2, 8, and 100 p.p.m. of lindane, and samples taken 2 weeks after lindane feeding ceased showed approximately 2, 4, and 50 p.p.m. From this experiment it is shown that lindane may be stored in fat for at least 6 weeks when there is a considerable intake of this insecticide in the diet.

EXCRETION OF INSECTICIDES IN MILK.—After it was reported by Howell *et al.* (1947) that cows sprayed with DDT secreted the insecticide in the milk, an extensive study of the problem was begun at Kerrville. In the early tests it was found that muzzling cows to prevent licking did not affect the degree of milk contamination. Cows sprayed with xylene emulsions of DDT secreted about the same amount of insecticide as those treated with the same concentration of a wettable powder.

During the summer of 1947 cows in a number of commercial dairies near Kerrville were sprayed with insecticides at 0.5-per cent concentration for horn fly control, and the same materials were applied at 5.0-per cent concentration as residual sprays in the barns for house fly control. Composite milk samples were taken from each herd before treatments were begun and at intervals throughout the summer and analyzed for the presence of insecticide. The results have been reported by Carter *et al.* (1949). Shortly after the treatments were applied, DDT and TDE appeared in the milk at concentrations of about 1 p.p.m. and in 2 or 3 weeks only traces of the materials could be detected. Usually at that time it was necessary to respray the cattle for continued control of horn flies. After treatments for an entire season DDT and TDE averaged about 0.6 p.p.m. in the milk. Because the organic-chlorine method is not very sensitive, it was not determined definitely whether or not cows treated with chlordane, toxaphene, and methoxychlor secreted the insecticides in their milk. The analyses did indicate that chlordane and possibly toxaphene were secreted in small amounts; the results with methoxychlor were more doubtful.

Claborn, Beckman, & Wells sprayed cows with 0.5 per cent of chlordane, toxaphene, and methoxychlor, using both xylene emulsions and wettable-powder suspensions. Three weeks later the sprayings were repeated. Analyses by the organic-chlorine method were negative. However, the insecticides could have been secreted to the same extent as is DDT or TDE.

The same authors also analyzed the milk from four commercial dairy herds treated with DDT and from three herds treated with TDE for horn fly control during the 1948 fly season. Each animal was treated with 1 or 2 quarts of a wettable-powder suspension containing 0.5 per cent of the toxicant. During the same summer the dairy barns were sprayed with 5 per cent emulsions of the same insecticides. Sometimes house flies became annoying when horn fly populations were low; so the insectides were applied only to the barns on those dates.

Milk samples were taken periodically throughout the fly season and, in general, the levels of DDT and TDE found confirmed the data reported by Carter and coworkers. However, when the barns alone were sprayed, the level of insecticide contamination in milk rose to about the same extent it did when the cattle were sprayed directly. A special test was therefore set up to determine the effect of spraying barns with DDT, as reported by Claborn *et al.* (1950).

When feed troughs were not covered, or

were not washed after the spraying, barn treatment alone resulted in contamination of the milk. When troughs were partly covered the contamination was moderate, and when they were completely covered or thoroughly washed contamination was negligible or existed only as traces. However, it seems unlikely that in practical fly-control operations users would always take the precautions necessary to avoid contamination.

LINDANE IN MILK.—Last summer 10 dairy barns in the vicinity of Kerrville were sprayed by Condron and Wells with 0.25-per cent lindane emulsions and suspensions to obtain residues of approximately 25 mg. per square foot. Milk samples taken before the spraying and periodically thereafter were forwarded to the Hooker Electrochemical Company for analysis. R. H. Kimball reported that all the samples were free of lindane. The Food and Drug Administration also analyzed duplicates of some of the samples, but found no lindane.

One Jersey cow was thoroughly sprayed with a 0.03-per cent lindane suspension and was also fed 100 mg. of lindane mixed with her feed just prior to the spraying. The cow was allowed to graze freely and was thus able to lick herself. Milk samples were taken before the spraying and at intervals thereafter. Milk taken about 7 hours after spraying and feeding of the insecticide was reported by Dr. Kimball to contain 0.6 p.p.m. of lindane. Milk taken approximately 24 hours after treatment also contained 0.6 p.p.m. of lindane, but milk samples taken 3, 5, and 7 days after treatment were negative.

In another test three cows were sprayed with lindane. One was treated with 0.1 per cent and muzzled to prevent licking, another was similarly treated and not muzzled, and the third cow was sprayed with 0.05 per cent of the insecticide and muzzled. Milk samples were taken before treatment and periodically thereafter. Analyses show that during the first 3 days after the spraying appreciable amounts of the insecticide were secreted in the milk (Table 2).

Table 2.—Parts per million of lindane found in milk of cows sprayed with lindane.

Days Between Spraying and Sampling	Cows Sprayed with 0.1% Lindane		Cow Sprayed with 0.05% Lindane
	Muzzled	Not Muzzled	Muzzled
Untreated	0	0	0
1	1.5	2.4	1.0
2	.6	.7	.3
3	.4	.4	.4
5	.1	.3	.2
7	.1	0	0
14	0	Trace	.5

SUMMARY.—Three years' studies on the effects of sprays and dips of DDT, TDE, methoxychlor, chlordane, toxaphene, lindane, and technical benzene hexachloride are reviewed.

All the insecticides, when applied to livestock as sprays or dips, were absorbed and stored in the fat. Data are given on the circumstances, extent, and duration of such storage. The insecticides for which sufficiently sensitive analytical methods have been developed were also found in the milk of treated cows.

The spraying of dairy barns with DDT or TDE for fly control resulted in milk contamination unless special precautions were taken.

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Exposure of House Flies to Residues of Certain Chemicals Before Exposure to Residues of Pyrethrum

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The application of adjuvants and pyrethrum separately to insects was reported in England by David *et al.* (1947). They found that sesame oil, sesamin, and N-isobutylundecylenamide showed synergistic action when applied to *Aëdes aegypti* L. 15 minutes before a pyrethrum spray was applied. The same synergistic action was discovered independently by Lindquist *et al.* (1947), who found that sesamin oil, N-isobutylundecylenamide, and piperonyl cyclonene were highly synergistic when applied to house flies from 30 seconds to 4 hours before an exposure to pyrethrum spray.

This paper reports first on the effect of exposing house flies to surface deposits of chemicals, some of which had been reported as synergistic and subsequently exposing them to pyrethrum residues at intervals (4 times) up to 48 hours. Fifty-one chemicals were tested by this method, most of them having previously been found to have some synergistic effect (Lindquist *et al.* 1949). A limited number of these chemicals were then combined with pyrethrum, and the residues tested on flies.

Methods.—Wide-mouth quart jars fitted with screen-wire lids were used as test chambers. The inside surfaces were uniformly coated with acetone solutions of the chemicals to be tested by rolling the jars between the hands until the solvent evaporated. The chemicals selected as possible synergists were applied to the jars at the rate of 40 mg. per square foot, and the pyrethrum, applied in separate jars, was used at the rate of 0.5 mg. of pyrethrins per square foot. The jars treated with the test chemicals were used for several days before being re-treated, but the pyrethrum was made up fresh for each day's test. Some of the chemicals left an oily film on the glass surface, which interfered with the test. Laboratory-reared house flies, *Musca domestica* L., 3 to 6 days old and about of an equal ratio of the sexes were used. Tests were made at room temperatures of 70° to 80° F.

About 100 flies were introduced into the treated jar for 5 minutes, and then removed to screen-wire holding cages. After another 5 minutes 10 to 15 of these flies were placed in the pyrethrum-treated jar and records made of the time required to obtain 50 per cent and complete knock-down. These flies were then removed to a clean holding cage to determine the mortality in 24 hours. At intervals of 4, 24, and 48 hours, lots of flies which had been exposed to the various chemicals were removed from their holding cages and similarly exposed to pyrethrum. As a check on the effectiveness of pyrethrum alone, one to three lots of flies were exposed to the pyrethrum residues only each day for determination of speed of knock-down. Tests with each chemical were replicated three to five times. All flies held overnight were supplied with food.

Containers of the same type were used in the second series of tests. These jars were coated with combinations of 0.5 mg. of pyrethrins and 5 mg. of the chemicals to be tested. They were tested for knock-down and mortality using 15 to 20 flies in each test.

Results.—Of the 51 chemicals that were tested for synergistic action, the following six were outstanding in speed of knock-down and high mortality (listed in order of effectiveness): Piperonyl butoxide, N-isobutylundecylenamide, sesamin concentrate, piperonyl cyclonene, the ethyl ester of piperonylic acid, and *n*-Propyl Isome. Not all of these materials were toxic in themselves to flies but all increased the speed of knock-down of the flies on subsequent exposure to pyrethrum residues and caused a greater 24-hour mortality than pyrethrum alone (Table 1). Of outstanding interest was the fact that the flies exposed to the chemicals 24 and 48 hours before contact with pyrethrum were knocked down quicker than those exposed to pyrethrins alone. Mortality was also greater in these same tests. It is apparent that the synergist produced some change in the flies which lasted up to 48 hours.

Table 1.—Effect of exposing house flies to residues of synergists and later exposing them to a pyrethrum residue. Dosage 0.5 mg. and the synergists 40 mg. (per square foot). Average of 3 to 5 tests.

Chemical	Time Between Treatments	Time Required for Knock-Down 50%	Time Required for Knock-Down 100%	Mortality After 24 Hours
		Minutes	*Minutes*	*Per cent*
Synergist Followed by Pyrethrum:				
Piperonyl butoxide	5 minutes	6.1	11.0	100
	4 hours	5.5	9.5	100
	24 hours	4.7	8.7	100
	48 hours	5.3	8.3	62
N-Isobutyl undecylenamide	5 minutes	5.0	10.0	100
	4 hours	5.8	9.8	92
	24 hours	5.7	9.7	82
	48 hours	5.5	13.0	75
Sesamin concentrate	5 minutes	5.6	10.4	96
	4 hours	5.7	10.0	91
	24 hours	6.0	11.4	66
	48 hours	6.0	10.0	91
Piperonyl cyclonene	5 minutes	4.8	10.0	99
	4 hours	5.2	10.2	62
	24 hours	4.7	9.0	97
	48 hours	6.7	11.7	82
Piperonylic acid, ethyl ester	5 minutes	5.2	10.5	55
	4 hours	5.5	10.2	70
	24 hours	6.0	9.7	78
	48 hours	6.0	9.2	93
n-Propyl Isome	5 minutes	6.2	13.0	81
	4 hours	5.7	13.0	71
	24 hours	5.5	14.0	77
	48 hours	—	—	—
Pyrethrum only	None	9.7	19.9	36

Of the 45 remaining compounds, 14 showed a slight or temporary synergistic effect, when tested by the method described and 19 showed no apparent synergistic value with pyrethrins to flies by this method of testing. Twelve were in themselves toxic to flies, but the synergistic property if present was obscured by the toxic action in these tests. The compounds in these groups are listed below.

Chemicals that gave only slight decrease in knock-down time, high recovery, and produced only a slight temporary effect on flies:

o-Aminobiphenyl
N-n-Amyl-*n*-butyranilide
Benzyl butyl ether
N-Isobutylcapramide
p-Chlorobenzaldehyde oxime
2-Cyclohexylidenecyclohexanone
N,N-Diethylpiperonylamide
N,N-Diisobutylpiperonylamide
Hydroxycitronellal dimethyl acetal
Isosafrole
Phenylcyclohexanone (mixed isomers)
Phthalic acid, methyl propyl ester
Piperonylidenemalonic acid, diethyl ester
Phthalic acid, di-*sec*-butyl ester

Chemicals that showed little or no synergistic action and no toxicity to flies:

p-Aminobenzoic acid, methyl ester
N-Amylbenzamide
Anisalacetone
o-Benzoylbenzoic acid, ethyl ester
Ethylene glycol bis(chloroacetate)
Chloroacetic acid, 2-phenoxyethyl ester
Benzoic acid, *p*-tolyl ester
1,3-Propanediol dicrotonate
N-Cyclohexyl-N-phenylpropionamide
Diamylnaphthalene
Dihydrosafrole
Diglycolic acid, dipropyl ester
1-Ethyl-1,2,3,4-tetrahydro-2-naphthol
Homophthalic acid, diethyl ester
Glutaric acid, bis-(2-methylbutyl) ester
Glutaric anhydride
Phthalic acid, isobutyl cylohexyl ester
N-Methyl-N-propionylanthranilic acid, methyl ester
2,4,7,9-Tetramethyl-5-decyne-4,7-diel.

Chemicals that alone were definitely toxic to the flies and synergistic properties, if any, were not apparent by the test method used:

N-*n*-Amylpropionanilide
Benzaldehyde, di-*n*-butyrate
α-Benzalacetophenone (Chalcone)
N-*n*-Butyl-*n*-butyranilide
Butyric acid, piperonyl ester
Chloromethyl phenyl sulfone
Dinitro-*o*-*sec*-butylphenol
1,2-Cyclohexane-dicarboxylic acid, di-*n*-butyl ester
2-Phenothioxycyclohexanol
N-Propionyl-1,2,3,4-tetrahydroquinoline
Thiocyanic acid, N-dodecyl ester
Undecylenic acid

The six best compounds and eight other chemicals selected from the groups that showed some toxicity or possible synergistic action were combined with pyrethrum in the same deposit. Table 2 gives the results of these tests. Piperonyl butoxide, sesamin concentrate, and N-isobutylundecylenamide were as effective with this combined treatment as they had been in the separate exposure tests. The ethyl ester of piperonylic acid, piperonyl cyclonene, and *n*-Propyl Isome were less effective. All except benzaldehyde, di-*n*-butyrate and α-benzalacetophenone (Chalcone) increased the effectiveness of pyrethrum as a knock-down agent. Most of them increased the 24-hour mortality.

DISCUSSION.—The flies pre-exposed for 5 minutes to residual deposits of six chemicals and later exposed to pyrethrum, were easier to knock-down and suffered higher mortality than flies exposed only to the pyrethrum residue. This effect lasted for as long as 48 hours after the initial exposure to the synergists. The pre-

exposure did not appear to injure the flies. Only when they came in contact with the pyrethrins was an effect greater than that produced by pyrethrum exposure alone apparent. These materials (shown in Table 1) were, therefore, judged to possess marked synergistic properties.

Hartzell & Scudder (1942) and Hartzell (1945) have shown that several synergists produce definite histopathologic changes in the central nervous systems and other tissues of house flies sprayed in a Peet-Grady chamber. Pyrethrum also produces distinct changes and when a combination of the two agents is used, the results are a summation of the effect of both. They used large enough dosages to obtain moribund flies for the histological study. They also found that where synergists were used alone at low concentration in a spray little or no effect on the tissues could be detected by histological studies. In the present work flies were exposed for 5 minutes to a residue of 40 mg. per square foot. Contact of the insect with the residue was primarily through the appendages. This short period of exposure may limit any tissue injury to localized areas. It is evident, however, that any tissue injury persists for at least 48 hours.

Table 2.—Results obtained when house flies were exposed to combined residues of 0.5 mg. of pyrethrum and 5 mg. of the test chemical per square foot. Average of 3 tests.

Chemical Combination:	Time Required for Knock-Down		Mortality in 24 Hours
	50%	100%	
	Minutes		*Per Cent*
Pyrethrins alone	12	26	41
Pyrethrins plus:			
Piperonyl butoxide	3	6.7	100
N-Isobutylundecylenamide	7	14	80
Sesamin concentrate	5	14	100
Piperonyl cyclonene	11	21	89
n-Propyl Isomer	12	23	82
Piperonylic acid, ethyl ester	8	19	48
Butyric acid, piperonyl ester	9	18	61
Phthalic acid, di-*sec*-butyl ester	6	15	27
2-Phenothioxycyclohexanol	6	14	66
N-n-Amylpropionanilide	6	12	44
1,2-Cyclohexane-dicarboxylic acid	5	12	63
N-N-Diethylpiperonylamide	9	16	99
Benzaldehyde, di-*n* butyrate	14	34	94
α-Benzalacetophenone (Chalcone)	13	39	29

SUMMARY.—House flies were exposed for 5 minutes to a residue of 40 mg. per square foot of chemicals selected as possible synergists and then after intervening periods of 5 minutes, 4 hours, 24 hours, and 48 hours were brought in contact with a pyrethrum residue of 0.5 mg. per square foot. Fifty-one chemicals were tested by this method and of these piperonyl butoxide, N-isobutylundecylenamide, sesamin concentrate, piperonyl cyclonene, the ethyl ester of piperonylic acid, and *n*-Propyl Isome, in the order given, were outstanding in increasing the effectiveness of pyrethrins. These chemicals decreased knock-down time and measured mortality compared with that obtained by exposing flies to pyrethrum only. No visual injury or toxic symptoms were apparent in the synergist-treated flies, but apparently the synergist caused some more or less permanent effect that lowered the resistance of the flies to pyrethrum poisoning. When flies were exposed to combined residues containing 0.5 mg. of pyrethrum and 0.5 mg. of adjuvants, piperonyl butoxide, N-isobutylundecylenamide, and sesamin concentrate showed the greatest synergistic properties.

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DDT in Milk Following Barn Spraying[1]

DONALD E. H. FREAR, W. N. BRUCE and A. C. RAGSDALE[2]

Laake (1949) has reported that cows in DDT-sprayed barns may secrete milk containing measurable amounts of this compound, even though no DDT was applied directly to the animals. This fact, coupled with the recommendation by Federal officials in 1949 that the use of DDT should be discontinued in dairy barns, has caused considerable interest in the subject.

To secure factual data on the levels of DDT in milk from cows housed and fed in barns sprayed with DDT for fly control, an experiment was set up during the summer of 1949. This involved eight dairy herds, four in Pennsylvania, three in Illinois and one in Missouri.

PENNSYLVANIA EXPERIMENT.—Four herds of cows, selected as typical of central Pennsylvania, were chosen for the study. Barns A, C and D were wooden bank barns, relatively old, but for the most part in good repair. In these barns one or more walls of the cow stable were masonry, the balance wood. Barn B was a new concrete block structure. A spray suspension containing 10 pounds of 50 per cent wettable DDT powder per 100 gallons was applied to all barns on the morning of July 20, 1949, while the cows were in pasture. An effort was made to secure complete coverage, and the result was a considerably more thorough application than that given by the average custom sprayer. The spray was applied with a high-pressure outfit, using a hand gun, and spraying was continued until dripping was apparent.

The barns varied considerably in size, but calculating from the area and gallonage used, the average deposit was approximately 200 milligrams of DDT per square foot on walls and ceilings. All mangers, water cups, forage and salt blocks were completely covered with heavy brown wrapping paper before spraying was begun, and these coverings were not removed for 15 minutes after spraying was completed. Since the day was warm and bright, practically all drippings had ceased by this time; however, it is possible that occasional drip from the ceilings did occur later.

In all cases, the dairy herds were returned to their barns on the evening of July 20, some 6 to 8 hours after the spray was applied. Composite 1-quart samples of the evening milk were collected on the dates shown in table 1. These were representative of the production of the entire herd. The samples were preserved with 2 ml. of formalin, and kept under refrigeration, until analyzed. Analyses were made in duplicate, using the procedure of Schechter *et al.* (1947). The results are given in table 1.

ILLINOIS EXPERIMENT.—The three large modern barns in the experiment were treated in the same general way as the barns used in the Pennsylvania experiment. An average deposit of 200 mg. per square foot was applied, using a spray suspension containing 40 pounds of 50 per cent wettable DDT powder per 100 gallons of water. In Barn E the cows faced outward, and only the areas above the mangers and the walls immediately in front of the cows were treated. In Barn F only the ceiling and walls behind the animals were treated; Barn G was completely treated with DDT.

These barns were sprayed on August 13, and the cows returned to the barns the same evening. Milk samples were taken on the dates shown in table 1. The samples were taken and preserved in the same way as those from Pennsylvania and were shipped to State College, Pa., for analysis.

MISSOURI EXPERIMENT.—Barn H in this experiment was sprayed with a suspension of 40 pounds of 50 per cent wettable DDT per 100 gallons of water on August 19, and again on August 30. The cows were placed in the barn on August 25, and were in the barn each day thereafter. In this barn the feed mangers and water bowls were not covered when the barn was sprayed on August 19, but were thoroughly scrubbed with a brush

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A portion of the cost of the analytical work was paid by the National Agricultural Chemicals Association.

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and flushed with water on August 24, the day before the cows were placed in the barn. When the barn was sprayed on August 30, the mangers and water cups were kept covered.

Milk samples were taken as indicated, and sent to State College, Pa., for analysis. The results of these analyses are given in table 1.

Table 1.—DDT (in parts per million) in milk following barn spraying with DDT suspension.

	Dates							
Barn	7/18	7/20[1]	7/21	7/22	7/23	7/27	8/3	
A	0.1	0.2	0.2	0	0.1	0	0.1	
B	0	0.2	0	0.9	0.1	0	0	
C	0	0.1	0	0.5	0.1	0	0	
D	0	0.2	0	0.3	0.3	0	0	
	8/9	8/11	8/13[1]	8/14	8/15	8/19	8/26	9/9
E	0	0	0.4	0.2	0.1	0	0	0
F	0.2	0	0	0.1	0.1	0	0.1	0
G	0	0	0.3	0.2	0.1	0.1	0.1	0
	8/27	8/29[2]	8/31	9/1	9/2	9/6	9/13	9/20
H	0.3	0.2	0	0.1	0	0	0	0

[1] Barn sprayed on this date.
[2] Barn sprayed on August 19 and August 30.

DISCUSSION.—From the results given in table 1 it is apparent that under the conditions of this experiment, a small amount of DDT may be found in the milk of dairy cows which have been housed, fed, watered and milked in barns sprayed with DDT. From the data presented, it appears that DDT may be present in the milk on the day of application of the spray, and for a few days thereafter. The maximum amounts were found in most cases 1 or 2 days following the barn spraying operation and, with one exception, did not exceed 0.5 part per million. Milk samples taken one week or later after spraying the barns showed insignificant amounts of DDT.

With the data available, it is not possible to state with certainty how the DDT got into the milk. It is quite obvious, however, that the DDT found in the milk on the day the barns were sprayed must have been the result of mechanical transfer, rather than through the animals' bodies, since the cows were not in the barn at the time of spraying, and were not returned to their stalls until immediately before the milking operation. In the samples taken the first day following spraying and subsequently, it is possible that two sources of contamination occurred: mechanical and physiological. It is not possible to separate these with the information available.

It should be noted that in the Pennsylvania experiment, which showed the highest amounts of DDT in the milk following the barn spraying, it is quite probable that significant amounts of DDT were deposited in the mangers and elsewhere from ceiling drip after the removal of the protective coverings. Because cows normally eat the feed in the mangers completely and then lick the manger surfaces, any deposits of DDT on these surfaces would be removed practically quantitatively by the animals.

The average amounts of DDT found in the milk samples were as follows: day of spraying, 0.2 p.p.m.; first day following, 0.1 p.p.m.; second day, 0.3 p.p.m.; seventh day, 0; and fourteenth day, 0. These are somewhat larger amounts than those reported by Harris *et al.* (1950), but it would appear that if ordinary precautions are followed, the danger of serious contamination of commercial milk supplies as a result of barn spraying with DDT is rather remote. Small amounts of DDT may be found in the milk for a few days following the barn spraying, but under practical conditions, the pooling of milk from one particular farm with that from many other farms at the creamery or milk receiving station would result in extremely low concentrations of the chemical in any given sample of market milk, since it is hardly possible that the barns of all suppliers in a locality would be sprayed at the same time.

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Leafhopper Transmission of Blueberry Stunt Disease

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A virus disease, known as blueberry stunt, has become a serious menace to the cultivated blueberry industry in New Jersey. The disease, first observed in 1928, was described and named by Wilcox (1942) when its virus nature was demonstrated by budding. Stunt disease is characterized by a general stunting of the bush with many short, slender twigs, small, abnormally colored leaves, an absence of new whips, and loss of production because of the failure of the plant to make a normal number of fruit buds.

With the proof of the virus nature of the disease, insect transmission was suspected. A search for insect vectors was instituted by the N. J. Agricultural Experiment Station in 1943 and has been carried on continuously by several workers since that time. In 1947, L. O. Kunkel of the Rockefeller Institute confirmed, by means of graft and dodder transmissions, the conclusion reached by Wilcox that stunt is caused by a virus.[3]

This paper summarizes the investigations showing that a complex of *Scaphytopius magdalensis* (Prov.) and *S. verecundus* (VanDuz.) is responsible for the transmission of blueberry stunt disease.

Search for Possible Vectors.—Though leafhoppers (Cicadellidae) were the first to be suspected as vectors, other Hemiptera, as well as a species of Coleoptera and of Thysanoptera, have been included in transmission tests. From casual observation and sweep-net collecting on cultivated blueberries, it would appear that the total leafhopper population is rather low. No adequate list of hemipterous insects attacking blueberries is available. Therefore, early work was, of necessity, confined to those species swept most easily and abundantly from blueberry plants. Regular, systematic sweeping of blueberries during 1945 and 1946 revealed that leafhoppers were numerous and that many different species were feeding on the bushes. In 1947 and 1948 the use of pear psylla traps, as described by Kaloostain & Yeomans (1944), revealed that numerous hemiptera were present in blueberry fields. These traps also showed relative abundance of all leafhoppers in different fields or parts of fields, and their peaks of abundance in the field, much more accurately than could be gauged by sweep-net methods. Seventy-five different species of Cicadellidae were collected from New Jersey blueberry fields by trapping. Many of these at least feed on blueberries and, therefore, had to be considered as possible vectors of blueberry stunt disease. Thirty-four of the more common species were used in transmission tests.

Transmission Tests.—During 1943 1944, and early 1945, insects used in individual vector tests were not pre-fed on diseased bushes, but were collected from diseased blueberry plants and transferred directly to healthy potted test plants.

With the exception of 1946, when more plants were exposed than had been anticipated, one check plant was maintained for every exposed plant.

There were no positive results from transmission tests in 1943.

Evidence of insect transmission was first obtained in the 1944 tests with miscellaneous insects which included *Empoasca fabae* Harris, *Graphocephala coccinea* (Forster), *Agallia constricta* (VanD.) and unidentified Cicadellidae. In this test, a cage was built around a diseased field plant which was sprayed thoroughly with nicotine sulfate. After a few days, four healthy plants and the insects were introduced into the cage.

All four of the healthy potted plants became diseased. Unfortunately not all insects introduced into the cage were known, and insects could have been present in the egg stage in any of the plants used and might have hatched out after they were sprayed.

There were no successful transmission tests in the 1945 season. During this season, specimens of the *Scaphytopius magdalensis* (Prov.) and *S. verecundus* (VanD.) were collected from blueberry and tested

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[3] Unpublished.

as possible vectors in two experiments. These species have not been tested separately because adults are not easily distinguishable.

During 1946, two series of tests were conducted, plants from a different source being used in each series. In the first series, known insects were introduced into cages containing a diseased bush and four healthy potted plants, a procedure similar to that used in the successful transmission test of 1944. One check plant of this series developed stunt in 1947, whereas none of the plants in cages with known insects developed the disease. In the second series, test insects were first pre-fed on diseased bushes and then confined to single healthy potted plants by means of celluloid cylinders or cloth cages in 40 tests. Of this series, three out of four plants exposed to colonies composed of *Scaphytopius magdalensis* and *S. vere cundus* developed stunt disease, two in 1947 and one in 1949. None of the seven check plants of this series or plants exposed to other insects developed the disease.

Since none of these test plants were protected from insect feeding prior to or following the tests, and because of the few check plants maintained for this series, these results were felt to be indicative only.

Beginning in 1947 it became possible to extend the work with improved facilities. First of these improvements was erection of a large screen house for holding plants under insect-free conditions before and after tests. Forty-six different insects in 200 exposures were tested for possible vectors in 1947, and 35 insects for 317 tests in 1948. The majority of the tests these 2 years were with Cicadellidae, though Fulgoridae, Membracidae, Cercopidae, and Miridae were used in several tests. Because of the positive results with *Scaphytopius magdalensis* and *S. verecundus* combinations in 1946, tests were concentrated on these leafhoppers in hopes of proving conclusively their role as vectors. Out of 200 tests begun in 1947, 40 were with these species; and of the 317 tests in 1948 they were used 103 times.

Plants from the 1947 tests were kept in the screenhouse for the whole growing season and following winter. They were then planted during the spring of 1948 in a field isolated by a mile of pine woods in every direction from other cultivated blueberries. As a result of unfavorable growing conditions, no definite stunt disease symptoms developed in these plants in 1948, though three appeared to be possibly diseased by late summer of 1948. During 1949 these three plants and one other showed definite stunt disease symptoms. These had all been exposed to colonies composed of *Scaphytopius magdalensis* and *S. verecundus*[1] that had been pre-fed on diseased bushes. None of the 290 check plants or the 160 plants exposed to other insects have developed the disease to date.

The 1948 test plants were handled similarly and were planted in the same isolated field during the spring of 1949. Growing conditions for the plants, though not ideal, were much more favorable than those in 1948. By the latter part of September, 1949, 10 plants of the 1948 series exposed to pre-fed colonies combining *Scaphytopius magdalensis* and *S. verecundus* were diseased. None of the 214 plants exposed to other leafhoppers were diseased, nor were any of the 217 check plants.

All of the 17 positive transmission tests with known insects have been with *Scaphytopius magdalensis* and *S. verecundus*, although one of the plants of the 1947 exposure series that developed stunt disease was exposed to a mixed population of *S. magdalensis-S. verecundus* and *Gyponana* sp. Since there have been 37 tests with pre-fed *Gyponana* sp. since 1945 with no transmission, it appears probable that transmission in the case of this plant exposed to the mixed population was not caused by *Gyponana* sp. Table 1 summarizes all transmission tests. Thus out of 147 exposures to colonies of *Scaphytopius magdalensis* and *S. verecundus*, 17, or 11 per cent, resulted in development of stunt disease. At the same time 514 check plants kept free of insects and 410 plants exposed to other pre-fed insects all failed to develop the disease.

DISTRIBUTION AND DESCRIPTION OF THE VECTOR.—*Scaphytopius magdalensis* (Prov.) is distributed throughout eastern United States and eastern Canada. Though present as far south as Florida and Georgia, it is essentially a northern insect and can be expected wherever its

[1] Determined by P. W. Oman, U.S.D.A. Division of Insect Identification.

Table 1.—A list of insects to which blueberry plants were exposed in stunt disease transmission tests during the years 1943 to 1948 inclusive, and the number of plants exposed to each species.

	Total Plants Exposed	Total Positive Transmissions
CICADELLIDAE		
Agallia constricta VanD.	53	0
Agalliopsis novella (Say)	5	0
Aulacizes irrorata (Fab.)	5	0
Chlorotettix sp.	4	0
Draeculacephala sp.	29	0
Empoasca fabae Harris	20	0
Erythroneura sp.	10	0
Graphocephala coccinea (Forst.)	66	0
Graphocephala versuta (Say)	12	0
Gyponana sp.	37	1?
Kolla bifida (Say)	7	0
Macrosteles divisa (Uhler)	96	0
Neokolla gothica (Sig.)	4	0
Paraphlepsius irroratus (Say)	16	0
Scaphoideus sp.	4	0
Scaphytopius angustatus (Osb.)	6	0
Scaphytopius frontalis (VanD.)	5	0
Scaphytopius magdalensis (Prov.) *Scaphytopius verecundus* (VanD.)	149	17
Scleroracus sp.	5	0
Scleroracus vaccinii (VanD.)	4	0
FULGORIDAE		
Ormenis septentrionalis (Spinola)	3	0
Acanalonia bivittata (Say)	5	0
DERBIDAE		
Cedusa sp.	4	0
MEMBRACIDAE		
Cerasa sp.	4	0
Entylia sp.	5	0
Stictocephala lutea Walker	11	0
CERCOPIDAE		
Clastoptera saint-cyri Prov.	14	0
Philaenus leucophthalmus Lin	37	0
MIRIDAE		
Adelphocoris rapidus (Say)	4	0
Lygus pratensis (Lin)	9	0

The following insects were used with negative results in only 1 or 2 transmission tests:

HEMIPTERA
- CICADELLIDAE
  - *Acinopterus* sp.
  - *Agallia quadripunctata* (Prov.)
  - *Amplicephalus osborni* (VanD.)
  - *Dellocephalus* sp.
  - *Forcipata loca* DeL. & Cald.
  - *Graminella nigrifrons* (Forbes)
  - *Idiodonus kennicotti* (Uhler)
  - *Jassus olitorius* Say
  - *Norvellina seminuda* (Say)
  - *Parabolocratus major* Osb.
  - *Paraphlepsius collitus* (Ball)
  - *Paraphlepsius* sp.
  - *Polyamia* sp.
  - *Ponana* sp.
  - *Sanctanus* sp.
  - *Stirellus bicolor* (VanD.)
- ALEYRODIDAE
  - *Aleuroplatus* sp.
- MEMBRACIDAE
  - *Enchenopa* sp.
- CERCOPIDAE
  - *Clastoptera xanthocephala* Germ.
- FULGORIDAE
  - *Ormenis pruinosa* (Say)
  - *Phyloscellis atra* Germ.
  - *Scolops* sp.
- COCCIDAE
  - *Aspidiotus ancylus* (Putn.)

COLEOPTERA RHYNCOPHORA
- CURCULIONIDAE
  - Anthonomus musculus (Say)

host plants are found. *Scaphytopius verecundus* (VanD.) is a more southern species, not previously recorded north of North Carolina. In New Jersey nymphs and adults have been collected from several species of Ericaceae, namely, leatherleaf, *Chaemadaphne calyculata*, sheep laurel, *Kalmia angustifolia*, wild and cultivated highbush blueberry, *Vaccinium australe* and *V. corymbosum*, lowbush blueberry, *V. angustifolium*, huckleberry, *Gaylussacia* spp. sand myrtle, *Dendrium buxifolium*, and cranberry, *Vaccinium macrocarpon*.

One of the most striking characteristics of the genus *Scaphytopius* is the acutely angled vertex that has gained for the many species the common name of sharp-nosed leafhoppers. Adults of *S. magdalensis* and *S. verecundus* are differentiated from most other species of this group in the northeast by a dark face which is minutely irrorate, whereas the faces of the other common species that might be mistaken for them are light yellow or white. Hepner (1947) gives the following measurements for *S. magdalensis* males and females, 4.2 m.m. and 4.8 m.m. respectively; and *S. verecundus* males and females, 4.0 m.m. and 4.2 m.m. respectively. The slight differences in the size and color make it impossible for the field worker to differentiate between the two species with certainty. The nymphs of these species are easily identified by their sharp vertex and by the presence of a red or a black X-shaped mark on the back of the body.

HABITS AND LIFE HISTORY.—These species are most commonly found in low, moist locations which are also the natural habitat of their ericaceous host plants. Feeding is most commonly observed on leaves or on leaf-bearing twigs in the lower, shaded portions of the bush (Fig. 2). Eggs are laid only between the epidermal surfaces of the leaves of the host plant, the leaves chosen usually being on the lower portions of the bush.

In New Jersey these species have two broods. They winter in the egg stage in fallen blueberry leaves. Eggs start hatching in late April or early May. First generation adults appear in early June; the population reaches a peak in late June and early July. Nymphs of the second generation are present in the field from early July until late September; whereas adults of the second generation appear about mid-August, and their population builds up to a peak in late September and early October.

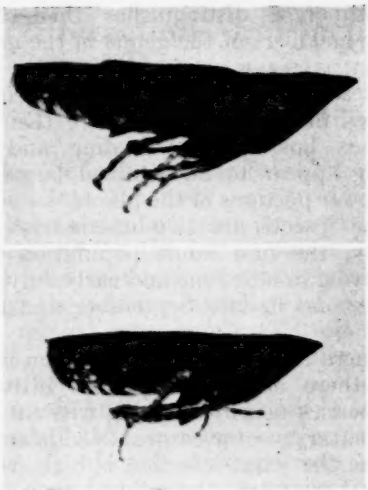

FIG. 1.—Adults of *S. magdalensis-S. verecundus* complex; female upper, male lower.

CONTROL.—Experimental control of *Scaphytopius magdalensis* and *S. verecundus* and of other possible stunt vectors has only recently been started. It is impossible, therefore, to tell yet how effective such a vector control program will be in arresting or slowing down the spread of the disease. When the initial stunt infection is low and there are no unrogued cultivated blueberries nearby, prompt roguing of diseased plants is an effective control. Where the stunt infection is rather high (5 per cent or more), roguing alone is probably impractical without supplementary vector control. The planting of resistant varieties may be practical in the future, but at present, only the Rancocas variety approaches complete resistance, and a few others such as Stanley and Harding, show a lesser resistance. No well-balanced planting should be confined, however, to these varieties.

During 1948 and 1949 eight plots in four blueberry fields were sprayed at 3-week intervals with 2 pounds of 50 per cent DDT or methoxychlor per 100 gallons of spray, and the reduction in leafhopper population was gauged by the comparative catches of leafhoppers on 10 pear psylla traps per sprayed plot and 10 traps for each of the eight comparable check plots. In the six sprayed plots and the six check plots that were located in young, non-bearing fields there was only a slight reduction of the leafhoppers that breed in blueberry fields, such as *Scaphytopius magdalensis-S. verecundus*, *Graphocephala coccinea* (Forster), and *Gyponana* sp. This was due to the fact that a large proportion of the leafhoppers present were migratory, because the young plants had not filled in the spaces in the row, and because of the small plots used (0.6 to 0.75 acre each).

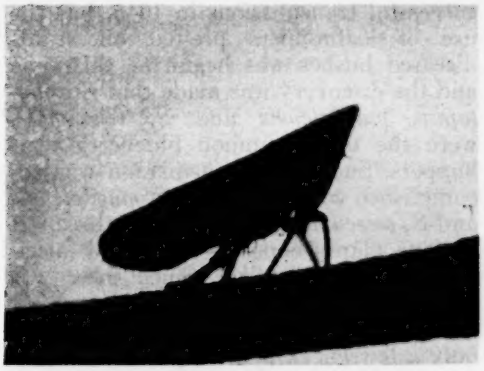

FIG. 2.—Sharp-nosed leafhopper feeding on young blueberry stem.

In the one mature field sprayed, both the spray and check plots comprised 2 acres each. The plants were heavily infested with leafhoppers, as the check trap catches indicated, and the migration factor was minimized by the size of the plots and almost complete ground coverage by the plants. In these plots there was a 95 per cent reduction in the sprayed plots of *Scaphytopius magdalensis—S. verecundus*, *Graphocephala coccinea* (Forst.), and *Gyponana* sp. as compared with the two check plots. Complete spraying of the entire field together with a strip of the surrounding vegetation should produce nearly perfect control in this area.

SUMMARY.—Blueberry stunt, a virus disease, has become a serious menace to the cultivated blueberry industry in New Jersey. Though Cicadellidae were suspected as vectors from the first, insects from other families and orders have been used in transmission tests. Regular sweeping and trapping in blueberry fields have revealed that a large and varied leafhopper population is present. Transmission tests were started in 1943. Successful transmission was obtained in 1944 with leafhopper colonies including *Agallia constricta* (VanD.), *Empoasca fabae* (Harris), *Graphocephala coccinea* (Forst.), and unidentified species. There were no

successful transmissions in 1945, but the use of leafhoppers pre-fed on stunt-diseased bushes was begun at this time and the discovery was made that *Scaphytopius magdalensis* and *S. verecundus* were the most common blueberry leafhoppers. Successful transmission was accomplished with colonies of *S. magdalensis* and *S. verecundus* in 1946 when three out of four plants exposed to pre-fed specimens became diseased. Since there was inadequate protection of the test plants before and after the tests, and because only a few cheek plants were maintained for this series, these results were felt to be indicative rather than conclusive. During 1947 and 1948 a total of 517 transmission tests were run, of which 143 were with colonies containing both *S. magdalensis* and *S. verecundus*. By late September, 1949, 14 of the 143 plants exposed in 1947 and 1948 to pre-fed colonies composed of *S. magdalensis* and *S. verecundus* had become diseased. None of the 417 check plants or the 374 plants exposed to other insects during these two seasons had developed the disease.

Scaphytopius magdalensis (Prov.) is distributed throughout eastern United States and Canada. *S. verecundus* is a southern species hitherto not reported north of North Carolina. Their hosts are various species of Ericaceae. A dark, minutely irrorate face distinguishes them from other members of the genus in the northeast.

These species are most commonly found in low, moist locations, as are their ericaceous host plants. Feeding and egg laying appear to be confined largely to the lower portions of the plant.

The insects are two-brooded in New Jersey, the first adult population peak occurring in late June and early July, and the second in late September and early October.

Where the initial stunt infection is low and there are no unrogued cultivated blueberries nearby, roguing without supplementary vector control is satisfactory. Where the stunt infection is high, vector control is a necessary adjunct to roguing. Planting only to those varieties showing resistance to the disease is not practical at the present time.

Spraying small plots of young plants with DDT has not controlled *Scaphytopius magdalensis* and *S. verecundus* because of migration into the small plots. In large plots of mature plants a 95 per cent control of *S. magdalensis* and *S. verecundus* has been accomplished. It is felt control could be made to approach eradication if the entire field and a border strip were sprayed.

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U. S. BUREAU OF ENTOMOLOGY & PLANT QUARANTINE CHANGES

F. R. LAWSON of the Division of Truck Crop and Garden Insect Investigations has transferred from the Twin Falls, Idaho station where he was engaged on the problem of development of vegetables resistant to insects, to the Oxford, North Carolina laboratory. He will investigate the biology of the green peach aphid as a pest of tobacco.

E. W. JONES, formerly stationed at the Walla Walla, Wash. station of the Division of Truck Crop and Garden Insect Investigations, was transferred to the Twin Falls, Idaho laboratory to assist in the development of control measures for the beet leafhopper. Mr. Jones had been engaged for 20 years on wireworm investigations.

H. C. MASON (Division of Truck Crop and Garden Insect Investigations) was transferred from Columbus, Ohio, where he had been investigating the biology and control of the tomato fruitworm and the Mexican bean beetle, to Beltsville, Md., to undertake a study on methods of preventing contamination by insects and insect debris in processed fruits and vegetables.

NEALE F. HOWARD, who had served for over 30 years in studying insects affecting vegetable crops in the Division of Truck Crop and Garden Insect Investigations, retired on August 31, 1949. Dr. Howard had been in charge of the Columbus, Ohio laboratory up until the time of his retirement.

Interactions Between Temperature and Toxicity of Four Insecticides Applied by Injection

NANCY WOODRUFF,[1] *Connecticut Agricultural Experiment Station, New Haven*

The application of insecticides by injection into the body cavity of insects has been a regular technique used in this laboratory for the last 3 years. It has been used to screen new compounds, to study the relative toxicities of nicotine and related compounds (Turner *et al.* 1948), and in many other studies of the action of insecticides.

Early in 1948, it was noticed in our experiments that an increasing percentage of water-injected controls were dying when stored at a constant temperature of 80° F. and 50 per cent relative humidity. This unexplainable loss of water-injected controls did not occur all at once, but has been noted in previous work (Turner *et al.* 1948).

Rhizopus, ordinary bread mold, was present in the petri dishes in which the bugs were held for observation. At the present time it is not known whether this mold can be related to loss in water-injected controls, but in order to reduce the mold, injected insects were stored in an ice box at 15° C. This procedure not only inhibited the mold, but stopped excessive mortality. It was decided to study temperature effect on the toxicity of several insecticides to see if any differential action would take place. Time of observation was extended from 48 hours to 7 days since the time required to kill might be greater at lower temperatures.

The effect of various temperatures on toxicity differs among different insecticides. DDT, for instance, is much less effective against houseflies at high temperatures than at low (Lindquist, 1945). Eagleson (1942), on the other hand, found that pyrethrins and lethane were more toxic to houseflies at high temperatures.

Potter & Gillham (1942), in testing contact poisons against *Tribolium castaneum*, showed that difference in environment before treatment did not affect toxicity, but that cooler conditions after spraying did increase the toxicity of such things as pyrethrins, lauryl thiocyanate, and DDT. It was their opinion that this increase in toxicity at lower temperatures was due to the physiological conditions of the insects themselves, since this effect occurred regardless of the nature of the chemical or the carrier used.

Jones (1933), in studying the relation of temperature to the toxicity of a soil fumigant, found that carbon disulphide at 32° C. was more toxic than at 7 degrees C., and that for every 10-degree drop in temperature the median lethal dose was doubled. In a study of respiratory metabolism and fumigants, Cotton (1932) showed that the susceptibility of an insect to a fumigant was influenced by anything which would accelerate the rate of metabolism, and that increase in temperature was one of these factors.

Very little work has been done relating effects of temperature to the action of injected insecticides into the body cavity of insects. Fan, *et al.* (1948) found a negative coefficient in the range from 15° to 20° C. when *Aëdes aegypti* larvae were dipped in DDT suspensions. With injection. however, this was not true, and it was their opinion that this difference was due to rate of adsorption through the cuticle which is greater at low temperatures. Hockenyos (1942), however, noted briefly that the speed of action of nicotine sulfate was more rapid at higher temperatures when it was injected into caterpillars. This type of application presumably permits the chemical in question to come in contact with its site of action more rapidly. This eliminates the problem of penetration of the cuticle which is so variable in different insects. It seems possible that any effect of temperature, therefore, may be more closely related to the chemical's toxic action, rather than on penetration or absorption through the integument.

Time of evaluation is a factor which cannot be ignored in any study involving temperature effects upon toxicity. The entire metabolism of insects is so much slower at low temperatures than at high, that response to any chemical may be proportional to the time at which it is observed, regardless of the nature of the application.

[1] Grateful acknowledgment is made to Dr. R. B. Friend, Mr. Neely Turner and Dr. R. L. Beard for their constructive criticism during the preparation of this paper.

Beard (1949) has shown that both the position and slope of dosage-response curves may change with differences in time of evaluation after treatment. He pointed out the importance of adequate studies so that the effectiveness of different insecticides could be compared with accuracy.

PROCEDURE.—In this study, four chemicals were used to test their specific action when injected into the blood of insects. They were nicotine, DDT, sodium azide, and rotenone. For the purpose of this paper, "fast-killing" will refer to a chemical which causes at least 50 per cent mortality within 24 to 48 hours, while "slow-killing" will refer to a chemical which kills slowly regardless of its immediate toxic effect. The four test compounds are classified as follows:

Nicotine	—slow-killing	high survival of paralyzed individuals
DDT	—slow-killing	low survival of paralyzed individuals
Sodium Azide	—fast-killing	high survival of paralyzed individuals
Rotenone	—fast killing	low survival of paralyzed individuals

All four chemicals were tested under uniform conditions in order to show the specific action of temperature and the time of evaluation.

Both nymphs and adults of the milkweed bug, *Oncopeltus fasciatus*, were used in this study. Injections were made with a Dutky-Fest apparatus (Dutky 1942), employing a standard tuberculin syringe and 27 gauge hypodermic needle. The delivery rate of the micrometer-operated injector was 3 microliters, which was the standard dose in all tests. The technique used was essentially the same as that described by Turner *et al.* (1948). All injections were made under ordinary laboratory conditions of temperature and humidity. All injections were made on insects previously anesthetized with carbon dioxide. Twelve bugs were injected with four concentrations of each material, the dosage ratio being the square root of two, and these were replicated four times. One series in each test was injected with distilled water which was the diluent used throughout the experiment, and stored at all test temperatures used. No attempt was made to separate the insects according to sex.

The injected bugs were placed in petri dishes containing a small amount of milkweed seed for food and a moist cellucotton plug for moisture, and stored in incubators at temperatures of 10°, 15°, 22° and 29° C. Two additional temperatures of 5° and 2° C. were also incorporated in this study. However, no survivors were recorded at these low temperatures with the exception of sodium azide-injected bugs. Therefore the effect of these additional storage temperatures will be discussed under that compound only. No attempt was made to control humidity since it was completely saturated within the petri dishes. For counting, all bugs were brought into the laboratory and were examined at room temperature. Observations of injected bugs were made daily for 7 days. The final, or seventh day count, was used as the end result unless otherwise noted. At this time, survivors at all temperatures were separated and saved for breeding purposes, the results of which will be published in a later paper on resistance. Normal oviposition occurred in all cases. The criterion of toxic action was as follows: dead—all individuals which showed no movement upon prodding and did not recover; paralyzed—obviously affected by the chemical and incapable of coordinated movement; active—affected individuals which were capable of normal activity.

Abbott's correction formula was applied to all data, but since it did not change the slope of the curves, and because control mortality is accounted for anyway, it was decided to present observed mortality and survival for all data alike.

EFFECT OF TEMPERATURE UPON INJECTION ALONE.—Mortality of water-injected controls, both nymphs and adults, increased with increase in temperature. Nymphs were more susceptible to the injection than adults as can be seen in table 1, Figure 1. This is not unexpected in view of the fact that the two stages of insect development are actually two separate physiological systems. One would expect more heterogeneity in nymphs of the species despite the fact that all those used were in the same instar. Some nymphs may have just completed one molt, while others may be preparing for the final one. Adults, on the other hand, were taken just after the integument had hardened, after the final

Table 1.—Per cent mortality of water injected controls at four temperatures.

	Temperature, Degrees C.			
	10	15	22	29
Nymphs	2	7.3	15.8	30.2
Adults	5	3	7.3	16.0

molt and before oviposition.

It is difficult to think of a clear-cut reason why increased temperature should affect the mortality of insects which have been injected with water. One can only speculate as to the reasons for these results because of the lack of data on what appears to be a physiological phenomenon. However, the introduction of any substance, even distilled water, by puncturing an insect's integument, is likely to affect the physiological system adversely. Further, exposure of the insect to an environment to which it is not accustomed may also be expected to produce harmful results.

Nicotine.—Nicotine, in the form of the free base, at concentrations of 0.4, 0.28, 0.2, and 0.14 per cent by weight, was injected ino both nymphs and adults of the milkweed bug. Since nicotine paralyzes immediately upon injection, we may assume that temperature would affect recovery rather than speed of action or penetration of the chemical.

Survival curves (Table 2, Fig. 2) at the four temperatures show that recovery was greatest at 29° for adults and 10° for nymphs. Adult survival increases with increment in temperature, whereas nymphal survival decreases. Also, adults appear to be more resistant to the effects of nicotine than nymphs, under the same conditions.

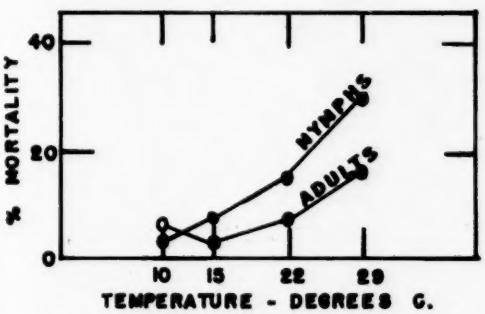

FIG. 1.—Arithmetic graph showing per cent mortality of water-injected nymphs and adults at four temperatures.

Table 2.—Per cent survival of nicotine injected nymphs and adults at four temperatures. (Final observation) average four replicates.

Per Cent Conc.	Temp., Degrees C.			
	10	15	22	29
	Nymphs			
.4	2	0	0	0
.28	8	2	6	0
.2	12.5	10	6	4
.14	33.5	27	25	21
	Adults			
.4	0	0	0	17
.28	4	4	12.5	46
.2	8	10.5	41.5	46
.14	27	46	60.5	58

The adult mortality curves (Table 3, Fig. 3), on the other hand, are not the exact reverse of the survival figures. The reason for this is the paralyzed bugs which are not included in the data given here. At low temperatures, the time required to kill is much greater than at high temperatures, and the seventh day count did not produce final results at low temperatures although it did at high temperatures. Therefore, the mortality curves actually measure, not final mortality, but response at each temperature for a given time interval. Because of this, dosage-mortality curves tend to flatten as temperature is increased. This difference in slope is also an indication that speed of action is greater at high temperatures.

In the case of nicotine, the time required for equal mortality of adults at a concentration of .4 per cent was 6.5 days

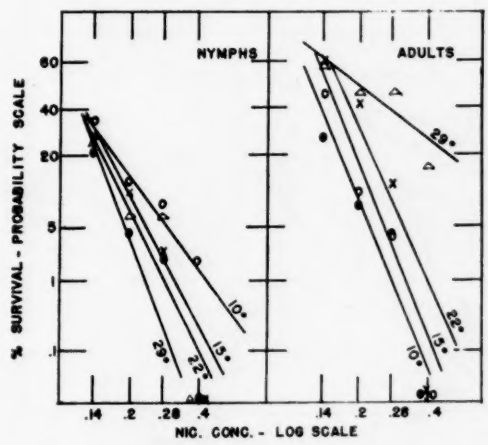

FIG. 2.—Log-probit survival curve of nicotine-injected nymphs and adults at four temperatures.

Table 3.—Per cent mortality of nicotine injected nymphs and adults at four temperatures. (Final observation) average four replicates.

Per Cent Conc.	Temp., Degrees C.			
	10	15	22	29
	Nymphs			
.4	40	54	77	90
.28	27	50	65	83.5
.2	10.5	21	67	80
.14	10.5	29	58	73
	Adults			
.4	27	34	19	52
.28	10	27	33	33
.2	6	20	15	40
.14	2	16	15	33

Table 4.—Per cent mortality of DDT injected nymphs and adults at four temperatures. (Final observation) average four replicates.

Per Cent Conc.	Temp., Degrees C.			
	10	15	22	29
	Nymphs			
1.0	54	79	98	98
.7	44	67	98	94
.5	29	58	94	81
.35	35	79	100	54
	Adults			
1.0	40	92	100	77
.7	37	81	98	77
.5	52	83	96	50
.35	35	85	73	33

at 10°, and 3.5 days at 29°. Obviously, time is important. However, the difference in slope between a curve for adult survivors at 29° and one at 10° indicates that temperature had an additional effect besides influencing the time required to kill the bugs. It is possible, of course, that a few more paralyzed individuals could recover, with time, but not enough to bring about the same percentage of survival at all temperatures. We must assume, then, that the highest temperature is directly responsible for an increase in survivors at all four concentrations.

It seems clear that with nicotine, nymphs and adults of the same species differ in their response to temperature, and that both must be compared in evaluating the toxicity of certain chemicals.

DDT.—A colloidal suspension of DDT, based on the Kido formula (Kido 1947), was used for injection at concentrations of 1, .7, .5, and .35 per cent by weight. The colloidal mixture, minus toxicant, injected into both nymphs and adults as controls, produced negative results.

DDT is also classified as slow-killing, but differs from nicotine in that very few paralyzed bugs recover. As has already been mentioned, Fan, *et al.* (1948) found a positive temperature coefficient for DDT action after injection, regardless of dosage.

In this study, however, substantial recovery of DDT-injected nymphs and adults of the milkweed bug took place at 29°, the highest temperature, which would indicate a negative temperature coefficient between 22° and 29° C.

Because it was felt that mortalities were too high for accurate interpretation of a log probit graph, an arithmetic graph (Table 4, Fig. 4) is presented which shows

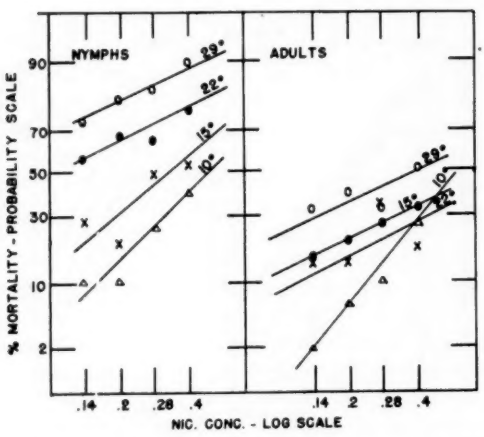

FIG. 3.—Log-probit mortality curve of nicotine-injected nymphs and adults at four temperatures.

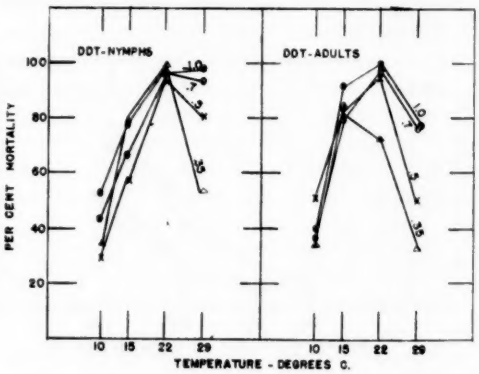

FIG. 4.—Arithmetic graph showing per cent mortality of DDT-injected nymphs and adults at four temperatures.

Table 5.—Per cent mortality of sodium azide injected nymphs and adults at four temperatures. (Final observation) average four replicates.

Per Cent Conc.	Temp., Degrees C.					
	2	5	10	15	22	29
	Nymphs					
.7	96	85	86	96	98	98
.5	79	69	67	69	86	96
.35	21	6	18	21	50	57
.25	0	10.5	12	12	54	48
	Adults					
.7	98	96	98	100	98	100
.5	81	85	96	96	89	94
.35	52	44	44	48	58	41
.25	10.5	17	23	52	46	41.5

Table 6.—Per cent survival of sodium azide injected nymphs and adults at four temperatures. (Final observation) average four replicates.

Per Cent Conc.	Temp., Degrees C.					
	2	5	10	15	22	29
	Nymphs					
.7	0	4	6	4	0	0
.5	0	0	25	27	6	0
.35	40	48	75	77	40	37.5
.25	79	64.5	88	87.5	44	52
	Adults					
.7	2	0	2	0	2	0
.5	2	6	2	4	8	6
.35	24	31	46	35.5	42	59
.25	64	73	64.5	48	50	58.5

as well as a log-probit curve, the relationship between temperature and mortality. Adults show a reduction in mortality at 29° at all four concentrations, while nymphs show reduction at only two. The dosage series for nymphs is probably too high. Death in the experimental insects increases as temperature is raised to 22° because the speed of action is so much faster at higher temperatures. Low temperatures slow down the speed of reaction of slow-killing chemicals, so that time of evaluation must be adjusted in order to produce equal results at all temperatures.

The increase in survival of affected bugs when stored at 29° may be specific for this species only. Nevertheless, injection produces a negative temperature coefficient between 22° and 29° C., and this must be a result of some other factor than absorption through the cuticle, which is not involved here. The higher temperature affected the physiology of the bug in such a way that it could withstand the toxic effects of DDT more easily than at low temperatures.

Sodium Azide.—Sodium azide is an inhibitor of oxidizing enzymes. It is readily soluble in water and was used at concentrations of .7, .5, .35, and .25 per cent by weight. It is not an insecticide in the ordinary sense, and was used in this study because of its specific nature. Sodium azide kills more rapidly than the other insecticides used in this study. There were very few paralyzed individuals at any time.

Inasmuch as azide is fast-killing at all

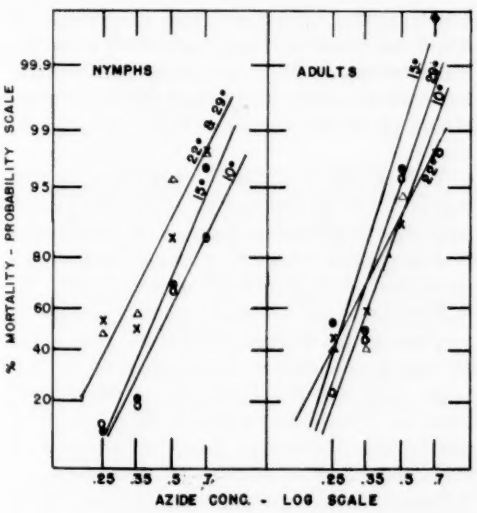

FIG. 5.—Log-probit mortality curve of sodium azide-injected nymphs and adults at four temperatures.

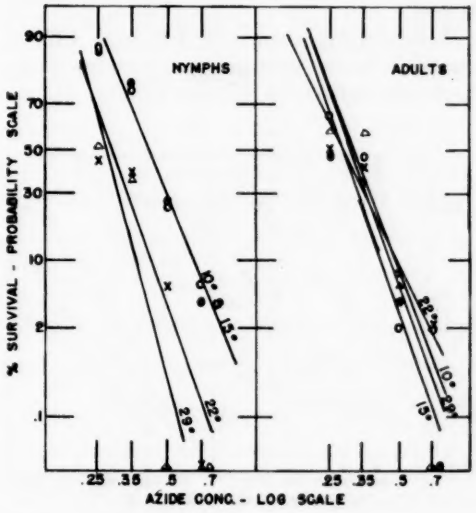

FIG. 6—Log-probit survival curve of sodium azide-injected nymphs and adults at four temperatures.

Table 7.—Per cent survival of rotenone injected nymphs and adults at four temperatures. (Final observation) average four replicates.

Per Cent Conc.	Temp., Degrees C.			
	10	15	22	29
	Nymphs			
.0175	10	0	2	0
.0125	2	4	0	0
.0087	19	4	4	0
.0062	8	19	0	0
	Adults			
.0175	2	6	0	0
.0125	6	4	4	0
.0087	4	0	6	0
.0062	12.5	14.5	15	14.5

Table 8.—Per cent mortality of rotenone injected nymphs and adults at four temperatures. (Final observation) average four replicates.

Per Cent Conc.	Temp., Degrees C.			
	10	15	22	29
	Nymphs			
.0175	85	98	98	100
.0125	79	86	90	100
.0087	71	75	81	98
.0062	79	77	92	92
	Adults			
.0175	96	94	98	100
.0125	88	92	96	100
.0087	83	92	92	100
.0062	69	71	79	86

temperatures, temperature effects are much less pronounced than with either DDT or nicotine. The mortality curves for adults show about equal kill at all four temperatures (Table 5, Fig. 5). All curves are steep and almost parallel. With nymphs, however, mortality is greater at 29° than at 10°. The slope of the mortality curve is very steep, indicating that there are relatively more survivors at the lower dosages than with the other materials. Unlike nicotine and DDT, azide is less effective against nymphs than adults. This might indicate that different enzyme systems exist in nymphs and adults.

With fast-killing chemicals, the time of evaluation factor is reduced considerably. (In these tests, final results of mortality and survival were observed at all temperatures).

Low temperatures of 10° and 15° C. enabled more nymphs to recover than high temperatures (Table 6, Fig. 6). At two lower storage temperatures of 5° and 2° C., recovery of sodium-azide injected nymphs was equal to that of 22° and 29° C. respectively. The temperature for optimum recovery of azide-injected nymphs was 10°. Further decrease in storage temperature did not produce additional recovery.

With adults, storage temperatures ranging from 2° to 29° C. had no effect on either mortality or recovery. Reversals such as this show the importance of the specific action of different chemicals, and the caution which must be used in comparing toxicities of different chemicals even within the same insect species.

Rotenone.—Rotenone was used as a colloidal suspension in the same manner as DDT. It was injected at concentrations of .0175, .0125, .0087, and .0062 per cent by weight. This chemical is extremely toxic to insects when injected into the blood stream. It is at least 20 times as toxic as nicotine, and about 50 times more effective than either DDT or sodium azide.

The mode of action of rotenone in killing insects is unknown. Tischler (1935) has reported that injection of derris into grasshoppers caused almost immediate cessation of respiratory movements.

Rotenone in some respects resembles sodium azide in its fast-killing properties at dose levels employed. However, in contrast with azide, a considerable proportion of rotenone-injected bugs became paralyzed. Recovery from this paralysis took place infrequently. In this respect it differed from nicotine paralysis from which many individuals recovered.

Mortality was so high at all four tem-

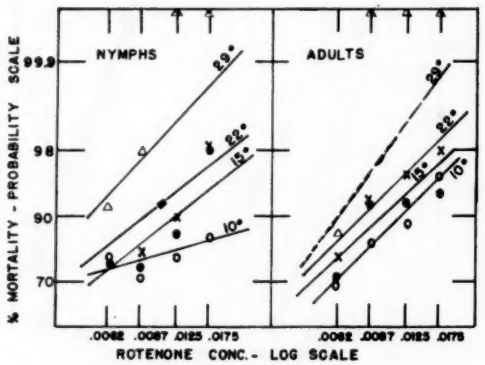

FIG. 7.—Log-probit mortality curve of rotenone-injected nymphs and adults at four temperatures.

peratures that it is difficult to interpret results. However, the toxicity of rotenone to both nymphs and adults was greater at 29° than at 10° C. The slope of the log-probit curve (Table 8, Fig. 7) is much flatter than the one for sodium azide. Only at the lowest temperature was there any appreciable recovery of either adults or nymphs (Table 7).

SUMMARY.—The effects of four temperatures on toxicity have been studied with four different chemicals injected into nymphs and adults of the milkweed bug.

Nicotine, a slow-killing chemical, was more toxic to nymphs than to adults. There were more adult survivors at 29° C. than at 10° C. and the reverse was true of nymphs. With both nymphs and adults, mortality increased with corresponding increase in temperature, up to 29° C. Higher temperatures bring about the end result of either death or survival more rapidly than low temperatures.

DDT, also a slow-killing chemical, produced a positive temperature coefficient between 10° and 22° C., and a negative temperature coefficient between 22° and 29° C. Recorded mortality of both nymphs and adults was low at 10° because most of the affected individuals had not died at the end of the observation period. Increasing temperatures up to 22° increased the mortality; the highest temperature, 29°, decreased it.

Sodium azide is a fast-killing chemical. With adults, about equal mortality was recorded at all temperatures. With nymphs, however, mortality was reduced at low temperatures. Nymphs were more resistant to azide than were the adults.

Rotenone, like sodium azide, was considered fast-killing at dose levels employed, and showed relatively no recovery of paralyzed individuals. High temperatures caused greater toxicity than low temperatures although mortalities were really too high for accurate comparisons.

CONCLUSIONS.—The general pattern of temperature effect upon the toxicity of several insecticides has been increased speed of action with increase in temperature. There is also some evidence of greater mortality at higher temperatures, with the exception of those chemicals which exhibit a negative temperature coefficient. It cannot be assumed that all insecticides have positive temperature coefficients. Once a coefficient has been established, it is obvious that a chemical need not be tested at all temperature levels.

Because of the mortality of water-injected controls at high temperatures, lower temperatures for storage after injection seemed to be desirable. Since the speed of action of most chemicals is much slower at low temperatures, time of evaluation must be adjusted in order to produce comparable data.

The action of nicotine upon nymphs and adults of the same species indicating opposite temperature coefficients is somewhat disturbing. The results of this investigation suggest the activities of separate physiological systems. The importance of the relationship of temperature to toxicity is also apparent.

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Properties of Acetylcholine Esterases from the Bee, the Fly and the Mouse and Their Relation to Insecticide Action

ROBERT L. METCALF and RALPH B. MARCH[1,2]

During an investigation of the relative toxicities and anticholinesterase actions of a number of derivatives of parathion, diethyl *p*-nitrophenyl thiophosphate (Metcalf and March, 1949) it was found that the compound di-isopropyl *p*-nitrophenyl thiophosphate was almost completely nontoxic to the worker honey bee, *Apis mellifera* L. and was a very poor inhibitor of bee brain ChE,[3] and yet was highly toxic to the female house fly, *Musca domestica* L. and was an active inhibitor of fly brain ChE. Such behavior was very different from that of parathion and its oxygen analogue, para-oxon (see Table 2), which were about equally active against both insects. This seemed to indicate that the combination of isopropyl and thiophosphoryl groups was in some way sterically incapable of combining with bee brain ChE. This hypothesis would suggest that there were specific differences in the protein structures of the true or specific ChE from the central nervous systems of flies and bees. Such differences had not been previously recorded in a study of specific ChE from several organisms (Augustinsson 1948). In view of the desirability for the development of insecticides nontoxic to beneficial parasites and predators, and of a comparatively low degree of mammalian toxicity, it seemed of importance to compare in detail the properties of ChE from the house fly, honey bee and white mouse.

MATERIALS AND METHODS.—The Warburg manometric technique was used for all ChE assays, as described by Metcalf & March (1949). Breis of bee heads, fly heads and mouse brains were prepared by homogenization in a buffer of 0.15M NaCl, 0.04M $MgCl_2$ and 0.025M $NaHCO_3$. The phosphate inhibitors were all prepared from purified compounds and added to the flasks in 0.1 ml. aliquots in acetone solution. The carbamate inhibitors were added in aqueous solution. Following addition of the inhibitors, the flasks were flushed with 95 per cent N_2 and 5 per cent CO_2 and allowed to incubate for 10 minutes. The acetyl choline, or other substrate solution, was then added from the side arm and the flask shaken for 5 minutes to reach equilibrium. The amount of CO_2 produced over a 30-minute period was then compared with a standard without inhibitor. Because of the slower rate of combination of di-isopropyl fluorophosphonate with ChE (Bain 1949) a reaction period of 4 hours was allowed before substrate addition. The values obtained at various inhibitor concentrations were plotted as probit of per cent inhibition vs. log. molar concentration to give straight lines from which the 50 per cent inhibition values were determined by inspection. In the determination of activity vs. substrate concentration curves, all values were corrected for non-enzymatic hydrolysis. In these determinations, a constant volume 0.3 ml. of substrate of the required concentration was used in the side arm of the flask.

The figures for median lethal dosage (LD_{50}) to the house fly and honey bee were obtained by the topical application of standard acetone solutions (March and Metcalf, 1949) and the oral LD_{50}'s to the male white mouse were determined by administration of propylene glycol solutions by the same method, or were taken from the literature as cited.

DISCUSSION OF RESULTS. *Relative Cholinesterase Activities in Bee, Fly and Mouse Brains.*—Values obtained for the relative ChE activities in bee, fly and mouse brains are given in table 1. The values for bee brain are the average of 19 determinations on separate breis, each containing 76 bee heads homogenized in 25 ml. of buffer; those for fly brain are the average of 10 determinations on separate breis, each containing 76 fly heads homogenized in 25 ml. of buffer; and those for mouse brain from 14 determinations on single whole brains. The values for mouse brain ChE, based on single brains rather

[1] Paper No. 647, University of California Citrus Experiment Station, Riverside.

[2] Pacific Slope Branch Paper, June, 1950.

[3] The following abbreviations will be used throughout: ChE for cholinesterase; ACh for acetyl choline; AMeCh for acetyl-beta-methyl choline; and BzCh for benzoyl choline.

Table 1.—Comparative ChE activity of bee, fly and mouse brains at 37° C., 0.02M ACh.

Tissue	Average Brain Weight	Microliters CO_2 per Brain per Hour	Micromoles ACh Hydrolyzed	
			Per Brain per Hour	Per Milligram Brain per Hour
honey bee female	2.5 mg.	127±3.2 (initial) 166±1.7 (24 hr.)	6.3± 0.6	2.5
house fly female	0.3 mg.[1]	76±3.8	3.4±0.17	11
mouse—male	400 mg.	3200±376	143 ±16.8	0.356

[1] Estimated from weight of intact head using same ratio of head weight to brain weight as found in honey bee.

than large numbers of pooled brains, are more variable as indicated by the high standard error. The high concentration of ChE in fly brains per mg. tissue is particularly noteworthy. This concentration of ChE is about 30 times that in mouse brains and is of about the same magnitude as the ChE concentration found in the electric organs of *Torpedo* sp. and *Electrophorus electricus* (Nachmansohn & Rothenberg 1945). The latter has been shown by Nachmansohn *et al.* (1941) to be able to split 1 to 3 times its own weight of acetyl choline in 1 hour, while the fly brain ChE in the experiments above split about 2 times its own weight of acetylcholine chloride at a substrate concentration at which the activity was later found to be less than optimum (see figure 1). No increases in ChE activity of brei from fly brains could be observed following overnight storage, although this always occurred with bee brains.

Comparative Action of Selected Phosphate Anticholinesterases.—The values obtained for 50 per cent ChE inhibition by certain phosphate anticholinesterases are given in table 2. The concentrations of central nervous system brei were adjusted to give fairly comparable ChE activities, and the following amounts were used per Warburg vessel: bee—8 heads or about 20 mg.; fly—8 yeads or about 2.4 mg.; mouse —50 mg. The data obtained are remarkable for the selectivity shown by diisopropyl *p*-nitrophenyl thiophosphate which was almost entirely inactive against bee brain ChE and mouse brain ChE, but highly active against fly brain ChE. The differences in activity were about 1000 times. The closely related diethyl *p*-nitrophenyl thiophosphate (parathion), di-*n*-propyl *p*-nitrophenyl thiophosphate and diethyl *p*-nitrophenyl phosphate (para-oxon) were of fairly comparable activity against the three enzymes. Tetraisopropyl dithiopyrophosphate, and the closely related tetra-isopropyl pyrophosphate are also both far more effective against the fly brain ChE than bee and mouse brain ChE's, while tetra-*n*-propyl dithiopyrophosphate and di-isopropyl fluorophosphonate are much more nearly of the same degree of activity to the three enzymes. The compound ethyl *p*-nitrophenyl thiobenzenephosphonate (EPN) is, in contrast, about 25 to 50 times as effective as an inhibitor of fly and bee brain ChE as it is of mouse brain ChE, and this difference in activity is correlated with lower mammalian toxicity. In general the relative toxicities of all of the compounds closely parallel their *in vitro* anti-enzyme activity. In attempting to explain these differences it seems possible that there must be specific differences in the protein structures of various true or specific cholinesterases from the central nervous systems of flies, bees and mice, for example.

Burgen (1949), Brauer & Pessotti (1949), Jansen *et al.* (1949) and Boursnell & Webb (1949) have provided ample evidence that these organic phosphate anticholinesterases inhibit ChE and other enzymes by combining with active centers in a 1:1 molar ratio; perhaps as suggested by Burgen by a phosphorylation of the enzyme by a dialkyl phosphoric acid, hydrolysis product. The data in table 2 would suggest stereochemical differences in the enzyme structures of bee, fly and mouse brain cholinesterases. Combination of the phosphate-ester with bee and mouse brain ChE appears to be hindered by crowding of the groups around the central phosphorus atom. This hinderance is evident with isopropoxy groups, com-

Table 2.—Relative anticholinesterase activity and toxicity of certain organic phosphate compounds to the house fly, honey bee and white mouse.

Compound	IN_{50} for Brain ChE[1]			LD_{50} in Mg. per Kg.		
	Fly	Bee	Mouse	Fly Topical	Bee Topical	Mouse Oral
1. diethyl *p*-nitrophenyl phosphate (para-oxon)	2.6×10^{-8}	1.9×10^{-8}	1×10^{-7}	0.5	0.6	3
2. diethyl *p*-nitrophenyl thiophosphate (parathion)	4.5×10^{-7}	1×10^{-6}	2.5×10^{-6}	0.9	3.5	6[2]
3. di-isopropyl *p*-nitrophenyl thiophosphate	2×10^{-5}	1.4×10^{-2}	$>1\times10^{-2}$	4.2	>1000	>100[4]
4. di-*n*-propyl *p*-nitrophenyl thiophosphate	5×10^{-6}	1.6×10^{-5}	3×10^{-5}	4	30	>100[4]
5. ethyl *p*-nitrophenyl thiobenzenephosphonate (EPN)	3×10^{-7}	8.2×10^{-7}	1.5×10^{-5}	1.9	3	50–100
6. tetra-isopropyl pyrophosphate	1×10^{-7}	1.5×10^{-5}	2×10^{-5}	6.5	23	50–100
7. tetra-isopropyl dithiopyrophosphate	3×10^{-6}	3×10^{-4}	1.5×10^{-3}	30	1000	>200[4]
8. tetra-*n*-propyl dithiopyrophosphate	5×10^{-7}	3×10^{-6}	5×10^{-5}	15	200	>200[4]
9. di-isopropyl fluorophosphonate	1.3×10^{-8}	2×10^{-7}	8×10^{-8}	15	30	37[3]

[1] *In vitro* molar concentration for 50 per cent ChE inhibition, 37° C., 0.02 M ACh.
[2] American Cyanamid Company: Manual of Experimental Data and Manufacturing Information for Thiophos Parathion.
[3] Horton *et al.* (1946).
[4] Limited solubility in propylene glycol.

pound 6; is increased by the combination of thiophosphoryl and isopropoxy groups, compound 7; and reaches a maximum with phenoxy, isopropoxy and thiophorphoryl groups, compound 3. This subject will be considered in greater detail in a subsequent paper.

Comparative Action of Carbamate Anticholinesterases.—The selective inhibition of fly and bee brain ChE by di-isopropyl *p*-nitrophenyl thiophosphate aroused interest in possible selectivity in the action of carbamate-type anticholinesterases. Hawkins & Gunter (1946) and Hawkins & Mendel (1949) have reported on the selective action of dimethyl-carbamate of (2-hydroxy-5-phenylbenzyl)-dimethylammonium chloride (Nu-683) which inhibited nonspecific or pseudo-ChE in dogs without affecting specific ChE, and on N-*p*-chlorophenyl-N-methyl-carbamate of *m*-hydroxyphenyl-trimethyl ammonium bromide (Nu-1250) which was from 100 to 1000 times as effective against specific ChE as against pseudo-ChE [see also Aeschlimann (1946) and Aeschlimann and Stemple (1946)]. The relative effectiveness of these two materials and of physostigmine sulfate (eserine) as inhibitors of bee brain, fly brain and mouse brain ChE's are given in Table 3. The relative concentrations of physostigmine sulfate and Nu-1250 required for 50 per cent enzyme inhibition of fly and bee brain ChE were very closely comparable. However, Nu-683 was about 100 times as effective an inhibitor of fly brain ChE as it was of bee brain ChE. Additionally, although Nu-683 was about 1000 times as effective an inhibitor of fly brain ChE as Nu-1250, the two compounds were about equally effective against bee brain ChE. However, with mouse brain ChE the converse was true and Nu-1250 was about 40 times as effective as Nu-683. This latter observation confirms that of Aeschlimann (1946) and Aeschlimann and Stemple (1946). The effects of the carbamic acid ChE-

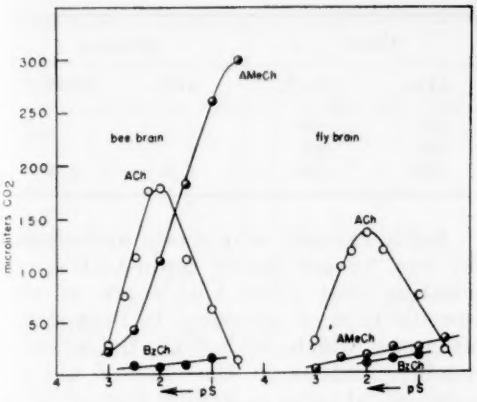

FIG. 1.—Activity-pS curves for enzymatic hydrolysis of ACh, AMeCh and BzCh by bee brain and fly brain ChE.

inhibitors are further indications of specific differences in the chemical properties of central nervous system ChE's from different animals. However, it seems doubtful if the compounds Nu-683 and Nu-1250 can be relied upon to distinguish between specific- and nonspecific-ChE's proposed by Hawkins & Mendal (1949).

Activity-Substrate Relationships for Bee Brain, Fly Brain and Mouse Brain ChE's.—Augustinsson (1948, 1949) has emphasized the importance of activity vs. substrate concentration curves in the characterization of ChE's, and has shown that for specific ChE these are bell-shaped, exhibiting pronounced inhibition with excess substrate. Richards & Cutkomp (1945) and Tobias *et al.* (1946) have called attention to the fact that bee brain ChE and nerve cord ChE from the American cockroach hydrolyzed AMeCh at a higher rate than ACh, in contradistinction to central nervous system ChE from mammals (Nachmansohn & Rothenberg (1945). Augustinsson (1949) has suggested that the investigations with insect ChE's were made at substrate concentrations well beyond the optimum for ACh, which would account for the low values obtained in comparison to AMeCh. Babers & Pratt (1950) found that fly brain ChE was more effective in hydrolyzing ACh than AMeCh. In order to resolve these rather conflicting viewpoints, activity vs. substrate concentration curves were plotted from data obtained with breis from 25 bee or fly heads homogenized in 25 ml. of buffer, or with a single mouse brain in 20 ml. of buffer and the breis

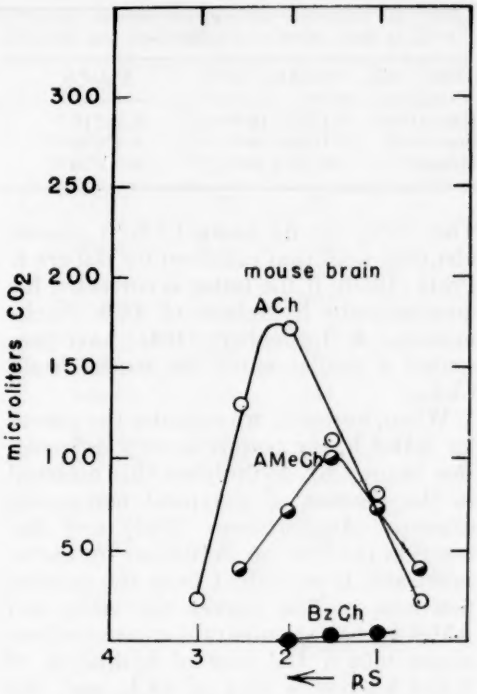

FIG. 2.—Activity-pS curves for enzymatic hydrolysis of ACh, AMeCh and BzCh by mouse brain ChE.

added to Warburg flasks at the rate of 2.6 heads, or 50 mg. mouse brain tissue per flask. Activity was plotted against varying substrate concentrations of acetyl choline bromide, acetyl beta-methyl choline chloride and benzoyl choline chloride, and the resulting curves were corrected for nonenzymatic hydrolysis. The results appear in figure 1 and 2 where activity over a 30-minute period is plotted against the negative log. of the substrate concentration (pS).

It is apparent from these curves that bee brain, fly brain and mouse brain ChE's all react with ACh to give the characteristic bell-shaped curves, exhibiting very similar optimum substrate concentrations close to 10^{-2}M ACh, and pronounced inhibition by excess ACh due to the formation of an enzyme-substrate complex, as postulated by Haldane (1930). The dissociation constants, Ks and the theoretical maximum reaction velocities, Vmax, of the enzyme-substrate complexes, calculated by the graphic methods of Lineweaver and Burk (1934), and the negative logarithms of the optimum substrate concentrations, pS, are as follows:

	Ks		Vmax		pS opt.	
	ACh	AMeCh	ACh	AMeCh	ACh	AMeCh
Fly	1.75×10^{-2}	3.7×10^{-2}	159	37	2	—
Bee	1.95×10^{-2}	2.4×10^{-2}	217	322	2	—
Mouse	2.58×10^{-3}	6×10^{-3}	235	120	2.3	1.5

The curve for fly brain ChE is almost identical with that obtained by Babers & Pratt (1950), if the latter is corrected for nonenzymatic hydrolysis of ACh. Nachmansohn & Rothenberg (1945) have presented a similar curve for mouse brain ChE.

When, however, we consider the curves for AMeCh, the picture is very different. Bee brain ChE hydrolyzes this material in the manner of a typical nonspecific esterase (Augustinsson 1949) and the reaction exhibits no inhibition by excess substrate. It is evident from the relative positions of the curves for ACh and AMeCh that at substrate concentrations above 0.02M the rate of hydrolysis of AMeCh exceeds that of ACh, and this apparently accounts for the observations of Richards & Cutkomp (1945), previously mentioned, who were working at substrate concentrations of 0.025M, at which point the hydrolysis of AMeCh is greater than that of ACh. Mouse brain ChE hydrolyzes AMeCh at a somewhat slower rate than ACh, but the curve has a similar shape and the enzyme is clearly inhibited by excess AMeCh.

Fly brain ChE is only very slightly active in hydrolyzing AMeCh, and the rate of hydrolysis is approximately equal to that of ACh, only at the very high substrate concentration of 0.3M. This is in good agreement with the data of Babers & Pratt (1950).

BzCh was only very slowly hydrolyzed by bee, fly and mouse brain ChE's, indicating that these ChE's are of the specific type as compared to nonspecific esterases which hydrolyze BzCh very readily, and as in the case of kidney esterase, at rates exceeding that of ACh (Nachmansohn & Rothenberg, 1945). Such nonspecific esterases do not appreciably hydrolyze AMeCh.

Hydrolysis of Mixtures of ACh and AMeCh by Bee Brain ChE.—The work of Augustinsson (1948) has shown that the activity of specific cholinesterases from dog brain or cow erythrocytes for ACh is inhibited by AMeCh and *vice versa*, and that choline is an effective inhibitor of ChE activity against ACh. In view of the atypical behavior of bee brain ChE with AMeCh, similar experiments were carried out with mixtures of these substrates, from which the data shown in figure 3A were obtained. These data clearly indicate that bee brain ChE will simultaneously hydrolyze both ACh and AMeCh without either substrate materially affecting the action of the enzyme on the other. This has been duplicated at a variety of substrate concentrations, as shown in Table 4. However, choline effectively inhibits the hydrolysis of either substrate. This apparently anomalous action is further evidence of the peculiar nature of the bee brain ChE. The most logical interpretation of these facts seems to be that ACh

Table 3.—Action of carbamate-type inhibitors on fly, bee and mouse brain cholinesterases at 37° C., 0.02M ACh.

	IN_{50}[1]		
	Fly Brain	Bee Brain	Mouse Brain
Physostigmine sulfate	2×10^{-8}	8×10^{-8}	3×10^{-7}
dimethyl carbamate of (2-hydroxy-5-phenylbenzyl) dimethyl ammonium chloride (Nu-683)	3×10^{-8}	3×10^{-6}	1.5×10^{-7}
N-*p*-chlorophenyl-N-methylcarbamate of *m*-hydroxyphenyl-trimethyl ammonium bromide (Nu-1250)	2.4×10^{-5}	4×10^{-6}	4×10^{-9}

[1] Molar concentration for 50% ChE inhibitor *in vitro*.

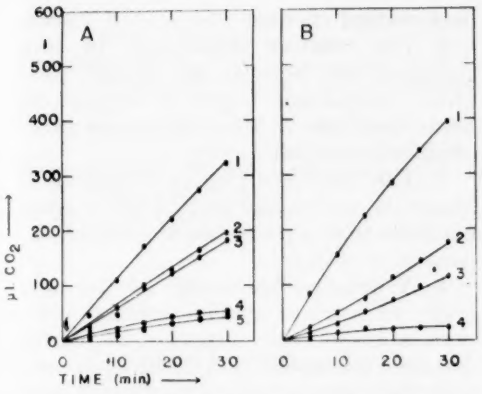

FIG. 3A.—Hydrolysis of mixtures of ACh and AMeCh by bee brain ChE. Curve 1. ACh 0.01M plus AMeCh 0.03M; 2. ACh 0.01M; 3. AMeCh 0.03M; 4. ACh 0.01M plus choline 0.03M; 5. AMeCh 0.03M plus choline 0.03M.

FIG. 3B.—Protective action of eserine against fly brain ChE inhibition by para-oxon with 0.02M ACh. Curve 1. control; 2. control plus eserine 2×10^{-8}M; 3. control plus para-oxon 3.3×10^{-7}M; 4. control plus eserine 2×10^{-8}M for 30 minutes followed by para-oxon 3.3×10^{-7}M.

and AMeCh are hydrolyzed at different sites on the ChE molecule, and that sites accessible to ACh are chemically different enough from sites accessible to AMeCh that no interchange is possible. However, both sites are accessible to choline. A rational explanation of such a phenomenon poses a problem similar to that of the inability of di-isopropyl *p*-nitrophenyl thiophosphate to combine with bee brain

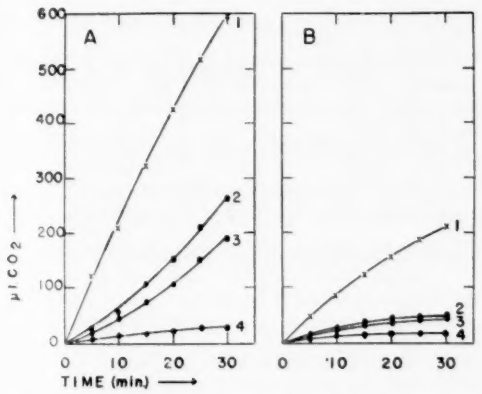

FIG. 4.—Protective action of eserine against bee brain ChE inhibition by para-oxon with ACh and AMeCh. A. Bee brain ChE with 0.02M ACh. 1. control; 2. control plus eserine 1×10^{-7}M; 3. control plus para-oxon 3.3×10^{-7}M; 4. control plus eserine 1×10^{-7}M for 30 minutes followed by para-oxon 3.3×10^{-7}M. B. Bee brain ChE with 0.02M AMeCh. Curves 1 to 4 with same reaction conditions as in A.

Table 4.—Hydrolysis of mixtures of ACh, AMeCh and choline by bee brain ChE at 37° C.

	Substrate	Substrate Concentration Molar	Microliters CO_2 in 30 Minutes	Per Cent of Competitive Inhibition
I.	ACh	0.01	170	—
	AMeCh	0.01	110	—
	ACh 0.01 AMeCh 0.01		270	3
II.	ACh	0.03	121	—
	AMeCh	0.03	169	—
	ACh 0.03 AMeCh 0.03		219	24
III.	ACh	0.01	188	—
	AMeCh	0.03	169	—
	ACh 0.01 AMeCh 0.03		351	2
IV.	ACh	0.01	188	—
	ACh 0.01 Choline 0.03		52	72
	ACh 0.01 Choline 0.1		40	79
V.	AMeCh	0.02	207	—
	AMeCh 0.02 Choline 0.03		42	80
VI.	AMeCh	0.03	169	—
	AMeCh 0.03 Choline 0.03		54	68

ChE, and its ability to combine readily with fly brain ChE.

PROTECTIVE ACTION OF ESERINE AGAINST BEE AND FLY BRAIN CHE.—*Inhibition by Para-oxon.* The protective or blocking action of eserine against ChE inhibition by DFP and TEPP has been studied by Burgen (1949) and Augustinsson & Nachmansohn (1949). These investigators have concluded that these agents act on the same active center of the enzyme. Similar studies repeated with fly and bee brain ChE have yielded the data shown in figures 3B and 4A and 4B. It is apparent that para-oxon and eserine are about equally effective in inhibiting the action of bee brain ChE on either ACh or AMeCh; and that pretreatment of the enzyme for 30 minutes with

eserine exerts considerable protective action against further ChE inhibition by para-oxon. The same general picture is true for the protective action of pretreatment of fly brain ChE with eserine against inhibition by para-oxon, where ACh is the substrate.

This information would indicate that para-oxon and eserine, like choline, are capable of combining with the centers of bee brain ChE active for the hydrolysis of either ACh or AMeCh.

SUMMARY AND CONCLUSIONS.—The following may be concluded from the data reported in this paper:

1. Distinct biochemical differences exist in the specific cholinesterases from the brains of house fly, honey bee and white mouse. These differences are reflected in (a) the relative anticholinesterase activity of organic phosphate compounds containing isopropoxy groups or aromatic rings or both or thiophosphoryl groups; (b) in the anticholinesterase activity of various carbamic acid esters; and (c) in the relative kinetics of cholinesterase behavior toward substrates such as acetyl beta-methyl choline.

2. The relative differences in anticholinesterase activity of organic phosphate compounds largely determine the acute toxicities of the compounds to the organisms studied.

3. The cholinesterase content of fly brains is extremely high, and is about equal to that reported in the electric organs of several eels.

4. A detailed knowledge of the properties of various cholinesterases and a correlation of the structure of various organic phosphate anticholinesterases with their specific action may result in the development of insecticides with a greater margin of safety to warm-blooded animals than those currently in use, and may enable the entomologist to select compounds toxic to certain insect pests, but relatively harmless to beneficial parasites and predators.

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The Oriental Fruit Fly: Progress on Research*

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The oriental fruit fly, *Dacus dorsalis* Hendel, was first observed in the Hawaiian Islands on May 10, 1946, in a collection of mango fruits made by Mabel Chong of the Territorial Board of Agriculture and Forestry. The fly was found to be well established at that time over the island of Oahu, and, as soon as surveys could be made, on all the other islands of the group also. It was clear then that the insect must have been in the islands for some time or was capable of developing huge populations and dispersing over large areas in a very short period.

A year ago, before this same group, H.M. Armitage (1949) described the problem from the mainland viewpoint. This paper followed the publication of a special report of the Joint Legislative Committee on Agriculture and Livestock Problems by the State of California on the Oriental Fruit Fly (1949). These papers, together with that of D. T. Fullaway (1949), might well serve as a more complete introduction to this discussion, since they summarize quite adequately the status of the pest as it was known at that time.

Then, the known host range included about 100 varieties of fruits and vegetables. The host list now includes over 125 varieties, many of which are of little economic importance and do not occur on the mainland. Economic losses can be incurred in three ways: first, by reducing grade and throwing into the cull class many fruits that have been disfigured by stings; second, by completely ruining the fruit and reducing it to a rotting mass; and third, by making necessary the establishment of restrictive quarantines. The problem from the quarantine standpoint has been recently described by C. E. Cooley (1950).

As soon as the seriousness of the situation was realized, many agencies in the Islands contributed to initial studies. The vast areas of wild hosts, particularly guava areas, made eradication out of the question. Direct control methods in these same wild areas were obviously impractical, and so it was natural that the first serious control efforts were directed to the introduction of parasites of *Dacus*. This work was started by the Territorial Board of Agriculture and Forestry in 1947 and was much extended in 1948, when a cooperative project was organized that added the resources of the Hawaii Agricultural Experiment Station, the Bureau of Entomology and Plant Quarantine, and the two private research institutions, the experiment station of the Hawaiian Sugar Planters' Association, and the Pineapple Research Institute, to those of the Territorial Board.

At this time also, the Fruit Fly Laboratory of the Bureau was actively engaged in the study of fruit flies and, with the advent of *Dacus dorsalis*, transferred most of its activity to study of the new pest.

The problem took on new importance to Hawaii when fruit fly eggs were found in flowers of *Vanda Miss Joaquim*. An embargo was immediately placed on mainland shipments of this flower, and for a time it looked as if this flourishing new industry would be completely lost to the Islands. The Fruit Fly Laboratory, however, met this situation and in a short time, on the basis of their work, ship-

* Presented at the meetings of the Pacific Slope Branch, June 1950.

ments were resumed after fumigation of the flowers with methyl bromide. This requirement was later eliminated.

In 1949 work on this insect was undertaken with funds appropriated by Congress in addition to Research and Marketing Act funds already allotted to Biological Control. It is with this development that this paper is primarily concerned.

THE ORGANIZATION OF THE ORIENTAL FRUIT FLY INVESTIGATIONS.—When a new insect arrives in an area and follows the typical pattern of such arrivals in a favorable physical environment and without biotic resistance, a research program must be envisioned that will attack the problem from as many angles as seems practicable. Initially, the approach to our problem appeared to offer possibilities along five separate lines: biological control, chemical control, area control, ecology-biology, and commodity treatments. A discussion of the objectives of these five projects might serve to clarify the basis on which research was initiated.

Biological control is too well understood to require much elaboration. In Hawaii, with some pests, this method of control has been singularly successful and, as has been mentioned, this project was being vigorously prosecuted before the current investigations were started.

Chemical control intends to explore all the possibilities for the control of the insect by insecticides, and by the use of lures and repellents.

Area control is more specific in its objectives and depends for some of its basic procedures on the Chemical Control project. Its purpose is to develop techniques whereby incipient infestations on the U. S. mainland could be contained and eliminated.

Ecology-biology has two objectives and perhaps these will explain the split term. The first is the effect of climatic factors on the species; the second, is the study of the biology and life history of the insect. Perhaps the terms synecology and autecology define the two aspects more clearly.

Commodity treatments have long been a major activity of the Fruit Fly Laboratory. It is concerned with the development of treatments which can be applied to infested fruits and vegetables so that they are safe to ship to uninfested areas.

The Investigations were organized, therefore, along these lines, and owing to the cooperation of the Washington office of the Bureau, the basic staff was assembled and operating by August 13, 1949. Shortly after this, the whole endeavor was strengthened by the coming together of all the agencies involved in a cooperative understanding with this Bureau. The following agencies are involved in this arrangement: the University of California, the Hawaii Agricultural Experiment Station, the Territorial Board of Agriculture and Forestry, the experiment station of the Hawaiian Sugar Planters' Association, and the Pineapple Research Institute. The California State Department of Agriculture, while not a formal signator to the Memorandum of Understanding, has nevertheless been a full member of the cooperative effort in every way.

EMPHASIS ON THE NEEDS OF MAINLAND AGRICULTURE.—It cannot be too strongly emphasized that by far the greater portion of the efforts of the entire Investigations, including those of the cooperating agencies, is intended for the benefit of mainland agriculture. It is hoped of course that the Biological Control project will give immediate relief to the local situation in Hawaii, but even this project is serving the mainland in a real way, for by reducing fly populations it will help reduce the opportunity for chance migrants to reach the mainland and will also provide a huge reservoir of beneficial insects that would be available for use in the event that a mainland infestation is discovered.

Commodity treatments also have an immediate value to local economy, although very few commodities actually exist in quality or quantity sufficient for export. The success of commodity treatments will no doubt influence the production of these agricultural commodities in the future, since up to the time of the first approved commodity treatment there has been little incentive among small farmers to produce for export. Even this project, however, will be of the greatest value to any infested area on the mainland from which fruits must be treated before shipment out of the infested area could be allowed.

The Chemical Control project is essentially a proving ground for old and new insecticides against this specific insect. Its data will be essential to orchardists and

other producers on the U. S. mainland should the fly reach there.

Area control is primarily and solely for the development of techniques to meet a mainland infestation.

The Ecology-Biology project has for its prime purpose the study of the effect of climate on the insect with the end in view of establishing a carefully based prediction of the possible distribution of the fly on the United States mainland. In the event of the development of an infestation, these data will serve for the establishment of safe areas and would contribute immeasurably to the free movement of commodities.

SECONDING OF TECHNICAL SPECIALISTS.—Material benefits have accrued from visits of specialists who have worked with the staff and familiarized them with special techniques. Randall Latta of this Bureau brought his long experience with methyl bromide fumigation and as a result fumigation chambers in the Territory are now operating much more efficiently and are subject to regular checks. R. L. Metcalf from the Citrus Experiment Station contributed materially to the techniques of Chemical Control. D. L. Lindgren of the same Station, has been with us for the last 6 months working on fumigation problems. Gordon Smith of the Division of Entomology and Parasitology at Berkeley has been assigned to each of the projects in turn over a period of almost a year. Cyril Gammon of the California State Department of Agriculture was released to join the staff as a full-time Bureau member and has brought an intimate knowlegdge of control operations to area control.

REPORTS.—It was recognized quite early that the usual procedures for disseminating information would not suffice, and so a system of regular quarterly reports was inaugurated. The first, covering the quarter ending September 30 essentially reported the organization of the Bureau's activities with initial data from all the projects being included. Since then these reports have been voluminous, but, since they serve as a repository of all the data of the Investigations including those of the cooperating agencies, this can be expected. Circulation is necessarily limited, but all the working agencies receive copies, and copies are also deposited in the libraries of the Division of Entomology and the Citrus Experiment Station of the University of California and of the State Department of Agriculture at Sacramento.

In addition to the formal reports, an informal monthly seminar is held, and the proceedings are distributed to all the participants. It is from these reports, which are expected to form the basis for many published papers under the authorship of the technical workers concerned, that the following summary of progress has been taken and which for convenience is described under each project heading.

The *Biological Control* project, with D. A. Clancy as leader, has the largest number of agencies, State and private, actively cooperating. The Bureau has provided increased facilities for reception and handling and for laboratory breeding, as well as additional explorers.

During the past year approximately 70,000 adult parasites of 36 species have been reared from 162 shipments containing 767,000 fruit fly puparia collected in Australia, New Guinea, Africa, India, China, Formosa, and the Philippines. These parasites include a great many species which could not be propagated on the fruit flies in Hawaii, but 6 species have been successfully bred and liberated in the field. Three of the parasites from Malaya are already established, two of these are now present in large numbers. They have increased and spread rapidly in many places and show great promise as control agents. Parasite collectors are being maintained in seven tropical areas, and it is anticipated that by the end of the next fiscal year all the possible sources of *Dacus* parasites will have been adequately explored.

Initially a project of this kind appears to express its results statistically with vast numbers of fruit fly puparia received and very large numbers of adult parasites emerging. Actually, of course, the problem is much more complicated than this. These parasites must all be tested under quarantine before they can be released for propagation. The urge to get parasites propagated and released also competes with time available for essential biological studies. However, a method has been developed for culturing fruit flies and their parasites on agar-base media. Pilot breeding, life history, and biological studies have contributed materially to our

understanding and utilization of these beneficial insects (Marucci & Clancy 1950, Bess, Van Den Bosch, Haramoto 1950, Mainland *et al.* 1950, Hardy 1949).

Propagation for release in the field is then taken over by the Territorial Board of Agriculture and Forestry, and releases are made at strategic points throughout the islands. The Hawaii Agricultural Experiment Station then takes over and studies the dispersal and recovery of these parasites and in addition is engaged on the difficult problem of evaluation.

The University of California is engaged in the development of techniques for the mass production of parasites under the direction of H. S. Smith. Findings to date in this project indicate that the large-scale production of the introduced parasites will be entirely possible.

Chemical Control, with L. F. Steiner as project leader, has broken much new ground and developed many new techniques and procedures. The discovery and development of methyl eugenol as a male lure for use in a low-cost type of trap in California will increase the effectiveness of mainland scouting operations by ten or more times that possible from the use of citronella, the best previously known lure. During the first 4 months' development of the methyl eugenol lure nearly two million male flies were captured in 30 glass traps. The chemical will attract males from 0.5 mile or more against a 10-m.p.h. wind. Where methyl eugenol was sprayed at weekly intervals onto the inside surfaces of box traps painted monthly with parathion, and placed 1/10 mile apart along the rims of gulches in pineapple areas, 650,000 flies were taken during the first 8 weeks, but only 80,000 during the next similar period. The percentage of males among the flies caught by fermenting lures in the vicinity declined from about 45 per cent before the first exposure of methyl eugenol to an average of 1.5 percent during the second 8 weeks and to zero in the final 3 weeks. This may prove to be an entirely new chemical means of control of an insect pest—a method innocuous to beneficial insects, since it would be limited to the trapping of males and consequent prevention of female fecundity.

Comparative tests under laboratory conditions have ruled out many common insecticides as being ineffective against the fly and have made possible a selection of the six or eight best for field testing. EPN,[1] dieldrin, parathion, and DDT were most effective as residual poisons, and since they are already under test on the mainland for other pests, mainland authorities will be able to put them to immediate use if the fly appears there; however, these materials have given less than 75 per cent control in small-plot field tests where unsprayed bananas averaged more than 100 larvae per pound. The finding that dieldrin, aldrin, chlordane, lindane, and *dilan*[2] are all more toxic than DDT in aerosol fogs will give a choice of space sprays for the immediate destruction of flies in any area where these toxicants can be utilized. The first two were ten times as effective as DDT, with lindane and chlordane four to five times as effective. No insecticide now known, however, has prevented all ovipositing flies from attacking the fruit, and an interesting relationship has been discovered between nutrition of the fly and its resistance to DDT.

Comparative tests in flights to and from Guam and on the ground, made with hand and automatic disinsectization equipment, favor adoption of the latter. The use of such equipment by commercial airlines should reduce the chances of hitch-hiking *dorsalis* reaching the mainland.

Development of an effective olfactometer for the more rapid screening of attractants should facilitate the development of female lures. This olfactometer has shown that females are also attracted by methyl eugenol at a high concentration, and this discovery may have a profound effect on future fundamental lure reasearch.

Area Control, with C. F. Henderson as project leader, might well have been deferred until Chemical Control and Ecology-Biology had acquired basic data. Actually, however, the initiation of all three projects at once, although producing some overlapping, has nevertheless given the Area Control project an opportunity to acquire a foundation on which to operate specifically for its own objectives. It was necessary first to establish various area categories such as urban, urban with

[1] Ethyl *p*-nitrophenyl thionobenzene phosphate.
[2] A 2:1 mixture of technical nitro-1,1-bis(*p*-chlorophenyl)-butane (CS-674A) and 2-nitro-1,1-bis(*p*-chlorophenyl)propane (CS-645A).

wild hosts in vicinity, and purely wild-host areas.

It was soon realized that the problems of reinfestation would make results difficult to interpret. Data were therefore obtained on fly movements into and out of the study areas, and from this data detailed studies were made of population densities and seasonal fluctuations. During these studies marked male flies have been recovered 20 miles from their original point of liberation. Other data on fly movement acquired throughout the Investigations have confirmed the migratory habit of the fly or at least its great capacity for dispersal. It has crossed an ocean strait 9 miles wide; it evidently moves back and forth over each island and possibly over more than one island; and it can be carried on the outside of fast-moving vehicles for long distances. This mobility will greatly complicate quarantine and control measures in the event of a mainland infestation.

An ideal method for the control of the insect in urban areas, which in Hawaii are rich in host fruits would be by the use of fog sprays. Tests with light doses of DDT applied in fog spray have proved promising. Dilute DDT sprays have reduced adult fly populations by 98 per cent. These tests have been made in villages on the windward side of Oahu, which are relatively free from reinfestation, each entire village being used as a sample control area. The use of many of the newer insecticides would greatly facilitate progress along these lines, and the data of the Chemical Control project are being constantly drawn on for this purpose. Public health considerations are important, however, and clarification of them will definitely open new possibilities in the use of fog sprays.

The island of Lanai is expected to provide the best large-scale experimental area in this project. No controls have been attempted there as yet. Instead, the year has been devoted to acquiring the basic data on which concentrated control measures will be developed. The entire island of approximately 89,000 acres, including a low, tree-covered mountain range, a model town full of host trees, large areas devoted to pineapple plantings, and still larger areas of lightly infested, almost-desert slopes, will provide an ideal location. The movements of the flies have been carefully followed; the host fruits wild and cultivated, are being mapped and their seasonal sequences plotted. Wild-host areas, principally guava on the lower mountain slopes, have been aerially surveyed to determine the feasibility of defoliation techniques that will eliminate the current crop of fruits. It can confidently be expected that during the coming fiscal year the Area Control project will be ready to throw its accumulated knowledge and resources against the fly on that island.

Commodity Treatments, with J. W. Balock as project leader, must continually face the basic difficulty of similarity between insect and plant protoplasm with respect to its susceptibility to treatment, and whereas many treatments that are lethal to the insect can be devised, the tolerance of the commodity is a real limiting factor. One successful treatment by vapor heat had already been approved for papayas. Since then this treatment has been extended to include tomatoes, bell peppers, and zuccini.

Modifications of the established vapor-heat treatment can only be established on the basis of long and repeated testing. During the year, as the result of such testing, information was developed that the supervised approach period could be eliminated and the treatment prescribed by regulation limited to the holding period, that is, the period during which the fruit is held after having reached the required temperature of 110° F. This procedure will materially facilitate handling and will improve the market quality of treated products. By reducing the time required for the all-over treatment, not only will local growers benefit, but also growers in any mainland area in which an infestation develops and from which fruit will need treatment.

The export of fresh pineapples has been made possible by establishing that a treatment, consisting of methyl bromide fumigation for 6 hours at 2 pounds per 1,000 cu. ft., will remove the hazard due to chance infestation of these fruits by fruit fly. This treatment has been a great boon to small growers in the islands who are once more in the business of producing pineapples for export as fresh fruit. Methyl bromide has also been used in extensive experiments on the treatment of papayas, tomatoes, and cucumbers, but although over 2,211 fruits with an estimated population of some 38,603 fruit fly eggs and

larvae have been used in these tests, the data are not considered sufficient to justify recommendations. More than 50 new fumigants have been screened for their value against the immature stages of the fruit fly, and the most promising ten of these have been selected for further trial.

In these tests the University of California and the Hawaii Agricultural Experiment Station have cooperated closely.

Entirely new methods for the treatment of infested commodities have not been neglected. Experiments with the Capacitron, a machine using accelerated electrons, have been carried to the point where the size and cost of the equipment have become a problem. A beginning has also been made in the use of new dielectric equipment which uses waves of high frequency. Tests with fruit flies can be made only if and when suitable equipment, which has already proved itself, can be made available in Hawaii; in the meantime the cooperation of Washington entomologists has been secured so that preliminary tests can be made with insects available on the U. S. mainland. An exception to this is the study of the effect of gamma rays. By cooperation with the Hawaiian Sugar Planters' Association experiment station a beginning has been made in this field, the experimental material being the eggs and larvae of fruit flies. The result from this experiment was interesting, not for the mortality obtained but for the fact that aborted flies emerged from the treated material.

Theoretically the incidence of infestation in commodities for export determines the severity of the treatment, but a valid procedure for determining incidence of infestation is a very difficult thing to achieve, and in actual practice commodity treatments are determined on such an extreme safety factor that the indices of infestation up to now have not really functioned in establishing these treatments. One example will suffice to illustrate the difficulties involved. A year's collection of papaya for index of infestation purposes showed 1 larva in about 4000 mature green papayas, which is the stage used for commercial shipping. From a recent collection, however, a single mature green fruit yielded 68 *Dacus dorsalis* pupae.

Ecology-Biology, with K. L. Maehler as project leader and N. E. Flitters as assistant project leader, has shown in life-history studies that the flies now have 128 host plants including practically all the economic fruits of the Islands and many California fruits. Some of these host records require careful evaluation. The fly develops such a pressure to oviposit that in the absence of more suitable hosts, or even in the absence of any hosts, it will deposit its eggs almost anywhere. Some host records are, therefore, to be regarded with caution, especially if the host involved is an economic plant. One such case developed in connection with the record of *Vanda* flowers as hosts. Eggs were found on these flowers and under very special circumstances the larvae developed. As a result, the flowers were embargoed and later released after fumigation. A long and careful study of the biology of the fly on this host, however, has shown that the commercial flower does not present any hazard to mainland agriculture; the documented evidence was considered by all those concerned and found unanimous acceptance. The fumigation requirement was therefore removed.

Age of sexual maturity has an important bearing on the success of chemical lures, and this project has determined that it takes 10 days for a male and 7 days for a female to become sexually mature after emergence; in the laboratory mating occurs at night and flies remain *in copula* for 2 to 12 hours, so that an important limiting factor may be low night temperatures below the threshold of sexual activity. This may well be the critical weak link in the biological chain, since it offers an opportunity for the elimination of the insect before maturity is achieved.

Hawaii offers a unique opportunity to study the effect of climatic factors on the ability of the fly to survive. Comparative altitude stations varying in elevation from 40 to 9,200 feet and with extremes of annual rainfall from 10 to 290 inches have been established on the islands of Maui and Hawaii. From these studies it has already been determined that at high elevations, with temperatures going below freezing, flies have survived a year. Flies may travel many miles in a short time. There is evidence of movement up and down mountain slopes being correlated with season. Under conditions of low temperature, the pupal period may be

extended from 9 to 60 days or longer. Eggs and larvae survive in fruit floated in fresh water for 7 days. When host fruits are scarce or limited, a low population will still result in heavy infestations in a good host. An isolated deciduous fruit orchard, situated on the slopes of Mauna Kea in an area believed to be climatically marginal, was infested with 1000 pairs of marked flies. These established themselves on a low population level, but survived the winter. Whether the survivors will be capable of initiating a new population remains to be seen.

Even with the wide range of altitude and climate available in Hawaii, it will still be desirable to test additional combinations of temperature and humidity to determine, in so far as these factors are limiting, the possible limits of distribution of the fly in the United States. Activity along this line to date has been concerned with instrumentation, and delivery is expected shortly on a series of cabinets which will enable us to reproduce with fair accuracy, the temperature and humidity curves of a hygrothermograph chart. We have called these instruments "bioclimatic" cabinets, but without any illusions concerning their limitations. It is manifestly impossible to attempt to control all the multitudinous aspects of climate and weather, but it is sufficient for our present purposes that temperature, and to a lesser extent humidity, are recognized and well-documented limiting factors in the distribution of insects. Finally, it has become clear that physiological studies of the fly are necessary to a proper understanding of its habits. Therefore, a new project is in the process of activation, and G. S. Fraenkel will shortly be available to advise on the setting up of the project, which will then be the responsibility of C. C. Roan.

To recapitulate briefly it can be said that real progress can be recorded in oriental fruit fly investigations, which are well supported, fully cooperative, and organized along broad and practical lines, with the protection of mainland agriculture as their main objective.

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DR. SIMMONS AND DR. HUGHES ATTEND WORLD HEALTH MEETING

Dr. S. W. Simmons and Dr. John H. Hughes, both of the U. S. Public Health Service, attended the meeting of the "Expert Committee on Insecticides" of the World Health Organization insecticide panel in Geneva, Switzerland, October 4 to 11, 1950.

DR. LEO KARTMAN GOES TO ARKANSAS

Dr. Leo Kartman has changed his address recently from the Johns Hopkins University School of Hygiene, Baltimore, Maryland, to Public Health Service, Malaria Investigations Station, Helena, Arkansas.

Control of the Pine Root Collar Weevil[1]

R. D. SHENEFELT, *University of Wisconsin, Madison*

The pine root collar weevil, *Hylobius radicis* Buchanan, has caused severe damage to Scotch pine in the central portion of Wisconsin, entire plantations having been killed in some cases. Where Norway or jack pine are adjacent to or intermingled with Scotch pine injury has frequently occurred on these trees also. A few infested Austrian and white pines have been observed. Dead areas are common in plantations and windbreaks composed of a mixture of Scotch and other pines. The weevil population builds up on Scotch pine and severe attacks may occur later on the other pines.

Damage is caused by the larvae which feed in the cambium region at the extreme base or in the larger roots near the trunk and eventually girdle the tree. Attack can be recognized by black masses of pitch, combined with soil, which contain the characteristic galleries. Often these masses are 3 or 4 inches thick. Schaffner & McIntyre (1944) gave a full description of the life history, distribution, habits and economic importance of the weevil. They found that ethylene dichloride with dinitro-o-cyclohexyl phenol or with dichloroethyl ether gave better control than other materials tested.

In the vicinity of Wisconsin Dells, an area noted for its recreational use, much Scotch pine was planted in the late 1920's and early 1930's for ornamental and recreational purposes. Many of these trees were killed by the weevil. During the fall of 1947 the writer was asked to visit the Crandall Plantation, a scenic plantation of Scotch, Norway and white pine established in 1926 near Wisconsin Dells by G. H. Crandall. About 1000 Scotch pines had been cut in a roadside area because of damage by the insect. Inspection revealed that the Scotch pine was heavily infested by the root collar weevil but adjoining Norway pine was still almost free from damage. The cutting was stopped and it was agreed to use a strip of 338 trees remaining in the center of the cut area for experimental purposes, with the remainder of the Scotch pine in the plantation to be treated with whatever appeared to offer the greatest promise of control.

After pruning the trees to a height of about 4 feet, emulsions were applied with a long-handled, pint-size dipper during the middle of June, 1948. One pint of emulsion was poured on the bark about 1 inch above the ground around the base of each tree. Some of the larger trees, however, required more than and some of the smaller ones less than a pint. Cost of trimming and application of the insecticide amounted to 7.3 cents per tree for the entire project. Untreated trees immediately across the road from the strip served as a check.

In October, 14 trees were examined in each of the treatments and in the check. Since the trees in the strip were to be taken out, it was possible to examine them by stripping the bark from the base and larger roots. Every tree in the strip showed outward evidence of having been infested. After peeling, a visual estimate was made of the percentage of actual girdling which had occurred. Numbers of living larvae, adults, other insects present and the condition of each tree were recorded.

Since the average diameters, including bark, of the trees varied and there were differences in the degree of infestation, the author believes that the most valid figure for purposes of comparison would be the relative number of live larvae or adults per inch of girdled cambium. Consequently the circumference and number of inches girdled was calculated for each tree. The numbers of living larvae found in the 14 trees examined for each treatment were averaged and then divided by the average number of inches girdled to obtain the number of larvae per girdled inch. The apparent percentage of mortality due to treatment was calculated by the following formula:

$$100-\left(\frac{\text{larvae/girdled inch in treatment}}{\text{larvae/girdled inch in control}}\times 100\right)$$

= apparent per cent mortality due to treatment.

Since only eight live adults were found

[1] Results of a cooperative project between the College of Agriculture of the University of Wisconsin and the Wisconsin Conservation Department. Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. G. H. Crandall, Inc. kindly furnished labor for the project and allowed use of trees on their property.

while the readings were being taken (and none of these were in the check) adults are not included in table 1, which summarizes the materials used and control obtained of larvae. Benzene hexachloride gave the highest degree of control.

During examination of the trees only one larva was observed with an external parasite feeding on it, but in a connecting chamber a parasite larva and a cocoon were observed. Nematode larvae, in masses, were numerous in the *Hylobius* galleries but no evidence was seen which would indicate that they were parasitic.

In addition to the above tests, 991 Scotch pines in blocks away from the road, *i.e.*, not in the strip referred to previously, were treated with benzene hexachloride emulsion because preliminary experiments had indicated that the material should be effective. While the trees in the strip were cut in 1949, those in these blocks were allowed to remain uncut. Examinations made in the fall of 1949 and 1950 showed that the infestation had been practically wiped out in the treated area but was still progressing at a rapid rate in the check area.

While it is not known how long the benzene hexachloride will remain in the soil around the base of the trees and afford protection, experiments made in connection with another project have indicated that the material retains its insecticidal activity in the same soil type for at least 2 years.

In 1950, 80 per cent of the Norway pine trees in windbreaks at a nursery in central Wisconsin were found to be infested with the root collar weevil. Treatment was made by using BHC at 1 lb. gamma per 1000 trees, using a power sprayer and applying about 2 quarts of water around the base of each tree with almost a solid stream from the nozzle. It was not necessary, in this instance, to trim the lower trunk or remove the litter.

From the standpoint of effectiveness of control, ease of handling and demonstrated persistence, benzene hexachloride appears to be the best material to use.

Table 1.—Results of tests for control of the pine root collar weevil.

Emulsion[1]	Average circumference	Average inches girdled	Larvae per girdled inch	Per cent mortality apparently due to treatment
Ethylene dichloride 25%	23.56	8.39	.085	80.2
Ethylene dichloride 25% plus DN[2]	24.91	5.49	.104	75.8
Ethylene dichloride 25% plus dichlorethyl ether 5%	25.0	5.90	.156	63.6
Propylene dichloride 25%	31.86	5.51	.026	93.9
Propylene dichloride 25% plus DN[2]	22.44	7.64	.112	73.9
Propylene dichloride 25% plus dichlorethyl ether 5%	26.82	5.14	.055	87.2
Chlordane (equivalent to 5 lb. actual/1000 trees)	24.16	8.67	.091	78.8
DDT (equivalent to 5 lb. actual/1000 trees)	24.91	12.91	.167	61.1
BHC (equivalent to 1 lb. gamma/1000 trees)	27.94	7.70	.009	97.9
Check	30.63	16.32	.429	0.0

[1] Materials supplied through the courtesy of Dow Chemical Company.
[2] 2-cyclohexyl-4,6-dinitro phenol 0.5 oz. per gallon.

The cost of trimming and application is, however, too great to allow other than high value trees, such as ornamentals or those in areas of high recreational value or windbreaks, etc., to be treated. Where Scotch pine is intermingled with other pines the cost is partially defrayed by the protection afforded the other species. Where it is possible to use power equipment for application the cost is greatly reduced.

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Toxicity of Spray Oils to Immature Forms of Citrus Blackfly

C. C. PLUMMER and J. G. SHAW, *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*

Investigations by Plummer & Shaw (1947) and by Woglum *et al.* (1949) have shown the high toxicity of finely ground cube root in emulsive spray oil to larvae and pupae of the citrus blackfly, *Aleurocanthus woglumi* Ashby. Since these studies did not indicate the effect of oil on the eggs, subsequent experiments, as described here, were performed.

TREATMENT OF EGGS.—A total of 155 small branches of new growth on sour lime trees growing in Cuernavaca, Morelos, Mexico, were trimmed to remove all except 12 to 15 terminal leaves, the branch directly below the leaves being left devoid of foliage. The terminal leaves were cleaned of all blackfly material with a small piece of cloth on November 7, 8, and 13 without damaging the leaves, and then covered with thin muslin sleeves. All sleeves were removed on November 13, 1947. By December 1 enough egg spirals were present to justify experimental spraying.

Terminal branches were sprayed on December 1 and 2 by means of a wheelbarrow sprayer at pressures close to 200 pounds per square inch. The pressure tank was removed to insure greater uniformity of mixing, and the emulsions were agitated constantly with a wooden paddle. The cloth sleeves were replaced as soon as the foliage had dried, usually within an hour.

The treatments included sprays containing 0.83, 1.25, and 1.67 per cent of light, light-medium, and medium emulsive oils.[1] The standard spray containing 1.67 per cent of light-medium emulsive oil with 4.5 ounces of finely ground cube root (5 per cent rotenone) to make 1 gallon was included, as well as untreated controls.

Twelve terminal branches with at least 10 leaves each were included under each of the 11 treatments. This design permitted the systematic picking of 4 samples of 5 leaves from each treatment. The leaves were examined under stereoscopic microscopes to observe the condition of eggs and to determine living larvae and pupae by the presence of body fluids when they were crushed with a needle.

Analysis of variance of the data from samples of lime leaves picked on December 16 and December 29 (330 leaves on each date) (Table 1) showed no significant difference between the number of egg spirals laid prior to treatment and none between the percentage hatch of eggs receiving different treatments. The first sample was picked before most of the eggs had hatched, to obtain information on the toxicity of oil to the older eggs. Actually, however, as will be shown later, oils are not toxic to eggs. The percentage of hatched spirals increased sharply in the second sample, but there was not a corresponding increase in the number of first-instar larvae on the leaves from treated terminals. In so far as there was a very high population of larvae in the controls, the deduction is that a high percentage of the larvae on treated leaves died. The addition of cube root to light-medium emulsive oil did not increase its toxicity when eggs were sprayed. The greater residual toxicity of medium oil to first-instar larvae is evident in the sample examined on December 29.

A third sample of 5 leaves was picked from each of 66 terminal branches on January 28 and February 4, 1948. Analysis of variance of data summarized in table 2 shows that there was no significant difference between the numbers of egg spirals on leaves prior to treatment. Practically all the eggs had hatched. The percentage hatch was surprisingly high, owing perhaps to some retention of moisture within the cloth sleeves surrounding the terminal branches. Large numbers of second- and third-instar larvae were found on leaves from control branches, and differences between the means for control plots and each treated plot are highly significant in all instances when tested by "t." These data show that greater larval mortality occurs in the second and later instars than in the first. There appears to be a trend indicating that the heavier the oil and the higher the concentration, the lower the survival of larvae.

Data from the fourth and last sample of 330 leaves picked on March 10, 1948, are summarized in table 3. Uniformity of numbers of egg spirals on leaves from different treatments was indicated by analysis of variance. The number of egg spirals hatching was generally high. The

[1] Characteristics of these oils are given in Calif. Dept. Agr. Spec. Pub. 233, pp. 79, 81, 83.

Table 1.—Effect on immature stages of the citrus blackfly of various emulsive oils applied on December 1 and 2, before the eggs hatched. Samples collected on December 16 and 29, 1947.

Grade and Strength of Oil Per Cent	Egg Spirals		Hatch of Eggs		First-Instar Larvae			
	Dec. 16	Dec. 29	Dec. 16	Dec. 29	Dec. 16		Dec. 29	
	Number	*Number*	*Per Cent*	*Per Cent*	*Number*	*Mean of* $\sqrt{n+0.5}$	*Number*	*Mean of* $\sqrt{n+0.5}$
Light								
0.83	642	296	37	87	371	7.03	244	6.08
1.25	626	540	24	94	512	8.66	323	6.19
1.67	483	391	22	86	202	5.65	223	5.94
Light-medium								
0.83	302	483	31	91	333	5.84	373	7.64
1.25	452	779	24	84	68	2.97	326	5.45
1.67	411	508	28	93	104	4.06	111	4.07
Medium								
0.83	459	483	17	90	311	5.72	111	3.80
1.25	389	446	22	95	154	4.43	53	2.51
1.67	452	287	29	94	63	3.28	81	3.17
Controls								
Light-medium oil plus cube	308	627	20	90	177	5.21	125	4.51
Untreated	350	566	27	79	703	9.57	2866	21.05
Significant difference between means:								
At 5 per cent level						2.01		3.73
At 1 per cent level						2.68		4.97

data shown support previous information in demonstrating the very high mortality of larvae and pupae from eggs treated with oil sprays, especially oils of light-medium and medium classification.

TREATMENT PRIOR TO OVIPOSITION.[1]—Adults of the citrus blackfly tend to congregate, often in large numbers, on the tender new leaves of citrus trees, and they oviposit on this foliage when it becomes a little more mature. In the prosecution of a control program it is important

[1] B. Peter Parachini and Francisco Servin assisted in these studies.

Table 2.—Effect on immature stages of the citrus blackfly of various emulsive oils applied on December 1 and 2, 1947, before the eggs hatched. Samples collected on January 28 or February 4, 1948.

Grade and Strength of Oil (Per Cent)	Egg Spirals	Hatch of Egg Spirals	Larvae of Indicated Instar			Pupae
			First	Second	Third	
	Number	*Per Cent*	*Number*	*Number*	*Number*	*Number*
Light						
0.83	382	98.5	1	21	42	0
1.25	614	96.8	1	13	27	0
1.67	538	97.7	4	11	0	0
Light-medium						
0.83	348	97.5	9	100	682	9
1.25	309	98.2	1	0	0	0
1.67	311	98.3	0	0	0	0
Medium						
0.83	536	98.7	0	1	1	0
1.25	317	96.3	0	0	1	0
1.67	352	98.7	0	0	0	0
Controls						
Light-medium oil plus cube	310	97.3	0	1	0	0
Untreated	372	99.8	77	1,242	3,853	41

Table 3.—Effect on immature stages of the citrus blackfly of various emulsive oils applied on December 1 and 2, 1947, before the eggs hatched. Samples collected March 10, 1948.

Grade and Strength of Oil (Per Cent)	Egg Spirals	Larvae of Indicated Instar[1]		Pupae
		Second	Third	
	Number	*Number*	*Number*	*Number*
Light				
0.83	306	1	8	55
1.25	289	0	0	27
1.67	345	0	0	1
Light-medium				
0.83	393	0	0	11
1.25	577	0	0	0
1.67	359	0	0	0
Medium				
0.83	405	0	0	0
1.25	470	0	0	0
1.67	224	0	0	0
Controls				
Light-medium oil plus cube	415	0	0	0
Untreated	381	64	154	1,533[2]

[1] No first instars found, except 4 in untreated controls.
[2] Includes 14 empty cases.

to know what happens to eggs laid on young foliage that has recently been sprayed with insecticides, particularly preparations containing oil, since they are the insecticides most commonly used for controlling this insect.

In November 1948 new growth and an abundance of citrus blackfly adults were found on Villafranca lemon trees growing in Ticuman, Morelos. Terminal branches with at least 10 leaves were carefully cleaned of infestation, and each was enclosed within a muslin sleeve. Eighteen blocks consisting of 6 terminal branches (plots) on the same tree were chosen. In a few trees it was necessary to select two blocks. Treatments were assigned at random. The terminal branches, including substitutions, were sprayed on November 16 with a wheelbarrow sprayer in the same manner followed in the first experiment, care being taken to protect unsprayed foliage and foliage treated with other insecticides. Emulsive spray oils at 1.67 per cent concentration were of the same source as those used in the previous experiment, including light-medium oil with 4.5 ounces of finely ground cube root (5 per cent rotenone) in enough oil to make 1 gallon.

After the clean foliage was treated, six terminal branches of each treatment were left uncovered, to expose them to infestation for 3 days. The remaining branches were closely inspected for freedom from infestation and were covered with cloth sleeves as soon as possible after being sprayed. Another group of six terminals in each treatment were exposed to infestation for 3 to 6 days, and a third group for 6 to 9 days after being sprayed. Living forms of the citrus blackfly were counted as before. The data from these treatments are summarized in table 4.

In both samples analysis of variance of the number of egg spirals laid on treated foliage showed significant differences between treatments, and highly significant differences between exposures. Furthermore, most of this variance took place in the 3-day or less exposure period, there being apparently enough oil on treated leaves to repel some flies from ovipositing on them. Thereafter, oviposition evidently took place in a normal manner since there were no differences. No statistical difference in the percentage hatch was evident between eggs laid on treated and on untreated foliage.

Both collections of leaves showed that many more first-instar larvae were present on leaves from control plots than on sprayed foliage and the difference between the control mean and each of the 4 treatment means is highly significant in the 3-day or less exposure period when tested by "t." Significant or highly significant differences were maintained in 6 out of 8 means in the 3 to 6-day exposure period. Differences of 2 out of 8 means in the 6 to 9-day exposure period were significant. The interpretation of these effects is difficult. It seems unlikely that residues of oil remaining on the foliage during an incubation period of 30 days would be sufficient to kill emerging larvae. Toxic effects might be exerted on eggs prior to hatching, and these effects might not be evident until after hatching had occurred. This would explain the lower survival of larvae from eggs laid on foliage up to 3 days after spraying, the greater survival of larvae from eggs laid 3 to 6 days after treatment, and much greater survival of larvae from eggs laid 6 to 9 days after treatment.

With the population of second-instar larvae differences between exposure periods are no longer significant in both samples. It is clear that by the time larvae reached the second instar the population in control plots far exceeded that of treated plots, the indication being that many larvae surviving the first instar died in the second instar. Differences between the higher control mean and the lower treatment means of each exposure

Table 4.—Effect on immature stages of the citrus blackfly of 1.67 per cent emulsive oils applied on November 16, 1948, before the eggs were laid. Sample 1 examined on January 4, 10, and 13, and sample 2 on February 15 and March 4, 1949.

Grade of Oil	Exposure After Spraying	Egg Spirals	Hatch of Egg Spirals	Larvae of Indicated Instar			Pupae
				First	Second	Third	
	Days	*Number*	*Per Cent*	*Number*	*Number*	*Number*	*Number*
			Sample 1				
Light	0 to 3	122	87	113	124	—	—
	3 to 6	201	48	103	105	—	—
	6 to 9	370	79	987	99	—	—
Light-medium	0 to 3	112	79	17	0	—	—
	3 to 6	266	83	453	168	—	—
	6 to 9	330	83	313	151	—	—
Medium	0 to 3	116	81	79	3	—	—
	3 to 6	125	72	5	1	—	—
	6 to 9	309	74	531	7	—	—
Controls							
Light-medium oil plus cube	0 to 3	92	88	56	8	—	—
	3 to 6	144	57	56	15	—	—
	6 to 9	306	78	581	14	—	—
Untreated	0 to 3	477	87	1780	1503	—	—
	3 to 6	248	81	973	561	—	—
	6 to 9	330	97	1521	1290	—	—
			Sample 2				
Light	0 to 3	58	90	36	3	23	6
	3 to 6	134	80	22	13	8	5
	6 to 9	373	100	24	25	40	149
Light-medium	0 to 3	56	100	0	0	0	0
	3 to 6	153	78	6	9	2	8
	6 to 9	348	96	8	6	19	45
Medium	0 to 3	85	72	7	0	0	0
	3 to 6	99	97	4	0	0	0
	6 to 9	81	92	0	1	0	0
Controls							
Light-medium oil plus cube	0 to 3	125	95	6	5	0	0
	3 to 6	140	80	0	0	3	0
	6 to 9	182	92	13	9	0	8
Untreated	0 to 3	397	97	162	324	822	811
	3 to 6	109	71	105	186	121	25
	6 to 9	229	84	48	147	99	66

group are highly significant in most instances when tested by "t." Differences between the means for various oil treatments within an exposure period are not significant when tested by "t." The first sample indicates that there are many more larvae surviving with light and light-medium oil than with light-medium oil and cube root or with medium oil.

Analysis of variance of the number of third-instar larvae and pupae in the second sample (Table 4) showed no significant difference between exposures and highly significant differences between treatments. There was no survival of larvae and pupae from eggs laid 0 to 3 days after spraying with light-medium oil, with and without cube root added, or with oil of medium weight. In fact, with medium oil there was no survival of larvae or pupae in any exposure period. For unknown reasons control populations are much reduced in the 3 to 6 day and the 6 to 9-day exposure periods.

SUMMARY.—In order to study toxicity to eggs of the citrus blackfly, small protected branches of sour lime trees with egg spirals of the citrus blackfly on the leaves were sprayed with emulsions prepared with 0.83, 1.25, and 1.67 per cent of light, light-medium, and medium emulsive spray oils. Light-medium oil at 1.67 per cent containing 4.5 ounces of powdered cube root (5 per cent rotenone) per gallon

of oil was also applied. Leaf samples showed uniformity of numbers of egg spirals prior to treatment. Oils were not toxic to eggs but a very high percentage of the emerging larvae did not survive. In some instances all larvae in the second and later instars failed to live. In general, the heavier the oil and the higher the concentration, the lower the survival of larvae. Toxicity was not enhanced by the addition of cube root to oil.

In another experiment uninfested foliage of lemon trees was sprayed with emulsions prepared with 1.67 per cent light, light-medium, and medium emulsive spray oils, as well as 1.67 per cent of light-medium oil containing 4.5 ounces of powdered cube root (5 per cent rotenone) per gallon, and then exposed for up to 3, 3 to 6, and 6 to 9 days, respectively, for infestation by the citrus blackfly.

Evidently there was sufficient oil on leaves for the first 3 days after spraying to repel some flies from ovipositing on them. There was no difference between percentage hatch of eggs laid on treated and untreated foliage. Fewer first-instar larvae emerging from eggs laid on foliage 0 to 3 days after spraying with oil survived than on the leaves exposed to infestation 3 to 6 days after spraying. Thereafter the effects of oil fell off sharply in the remaining 6 to 9-day group. Many larvae surviving the first instar died in the second and high populations were shown only in control plots.

Populations of third-instar larvae and pupae were more reduced on foliage that had been sprayed with light-medium oil, with and without cube root, than on foliage treated with light oil. No living third-instar larvae and pupae were found on leaves treated with medium oil prior to exposure to flies for oviposition.

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New Insecticides for Control of Onion Thrips in Southern California

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Investigations in southern California on the control of the onion thrips, *Thrips tabaci* Lind., have included tests on the effectiveness of several organic insecticides as compared with DDT. The insecticides were applied as dust mixtures with rotary hand dusters at approximately 30 pounds per acre per application. Randomized-block experiments were conducted in the Coachella Valley in 1948 on Crystal White Globe Wax onions and in the San Gabriel Valley in 1949 on Early Spanish onions.

DDT dusts and sprays have become standard treatments for the control of the onion thrips in most parts of the country. The development of these treatments has resulted from work by Lange (1946), Sleesman (1946), Ashdown & Watkins (1948), Wilcox & Howland (1948), and others. Chapman *et al.* (1947) reported control of this insect with benzene hexachloride on cotton, as did Fletcher *et al.* (1947). Hoerner & Edmundson (1947) reported good results with benzene hexachloride sprays on onions; Gaines *et al.* (1948) reported good control of the insect on cotton with toxaphene and benzene hexachloride, and in one test reported ineffective results with TDE, tartar emetic, and DDT. Fife *et al.* (1948) found toxaphene to be more effective than DDT alone or mixed with benzene hexachloride, and to be more effective than chlordane against onion thrips on cotton. Douglass & Shirck (1949) reported good results with sprays containing benzene hexachloride, chlordane, and toxaphene, and poor results with methoxychlor on onions.

EXPERIMENTS IN COACHELLA VALLEY.—In the Coachella Valley 2 fields were used. In the first field there were four replicate plots of weak dusts and four replicate plots of strong dusts. In the second field there were eight replicate

plots of the strong dusts. The weak dusts contained 1 per cent of parathion, 1 per cent of lindane, or enough benzene hexachloride to give 1 per cent of the gamma isomer; 5 per cent of DDT, TDE, methoxychlor, or chlordane; or 10 per cent of toxaphene. The stronger dusts contained the same insecticides but at twice the concentration. Western pyrophyllite was the diluent used in each dust. Two dust applications were made 2 weeks apart in each field and thrips counts were made on four plants per plot at 1- and 2-week intervals after each application. Applications were made in the first field on February 17 and March 4, and in the second field on March 25 and April 7. The plots in the first field were 50 feet long and 12 beds wide. In the second field the plots were 40 feet long and 12 beds wide. The beds in both fields were 26 inches from center to center and consisted of two rows of onions.

Thrips populations were not high, and with the effective materials control was considered to be satisfactory with the two applications, as the onions were harvested during May. With the weak dusts in the first field larval control ranged from 35 to 68 per cent and adult control ranged from 0 to 48 per cent. With the strong dusts larval control ranged from 48 to 86 per cent, and adult control from 35 to 71 per cent. In the second field where only the strong dusts were used, larval control ranged from 52 to 90 per cent and adult control from 33 to 56 per cent.

Adult thrips migrate readily, not only from the check plots but also from the untreated part of the field. For this reason, when small plots are used, the true effectiveness of the materials can be better determined by the results against the larvae, which do not migrate. In general, adult population is much lower than larval and, although there is some variation, the control pattern for adults is very similar to that for larvae.

The results for the two fields are shown in table 1. Chlordane, DDT, benzene hexachloride, lindane, and toxaphene gave better control of larvae than did TDE. Methoxychlor was inferior to all the other materials. In adult control chlordane, lindane, benzene hexachloride, parathion, DDT, and toxaphene were superior to methoxychlor and TDE.

Yields based on 20 feet of bed in the center of each plot are also shown in table 1. No significant difference was shown between treatments or between the treated and untreated plots because of soil variations within the field. There was a tendency, however, toward lower yields on the DDT plots than on some of the others treated with effective materials, and there was an increase in yield on the treated plots as compared to the check plots. The DDT seemed to affect the growth in such a manner as to delay the curing of the plants after irrigation was stopped. This feature is undesirable for early onions because they must be cured rapidly and marketed in a very short time. Lange & Thwaits (1945) noted this in experiments on late onions and said, "The fact that spraying onions with DDT would prolong their growth in the fall and retard their dying must be considered in spray operations involving this material."

The insecticide applications also tended to increase the bolting of the onions in the first field, but frost was the primary cause of bolting. Plants in the first field were transplanted early in January and were exposed to several severe frosts during that month. Those in the second field were transplanted in February after the last frosts, and no bolting occurred in this field. The percentage of bolting, based on 100 plants per plot, is shown in table 1. Although bolting was not in direct ratio to control, the least effective of the materials tended to have the lowest percentage of bolters. There was no significant difference in leaf height or number of leaves produced per plant between the various treatments.

EXPERIMENTS IN THE SAN GABRIEL VALLEY.—The 1949 experiments were conducted in two fields of Early Spanish onions in the San Gabriel Valley with 4 replicate plots in each field. The plots in the first field were 71 feet long and 8 beds wide. Each bed contained 4 rows of onions, and the beds were 32 inches from center to center. In the second field the plots were 31 feet long and 12 beds wide. The beds contained 2 rows of onions and were 32 inches from center to center. Dust applications were made at about weekly intervals, on May 24, June 1, and June 9 in the first field and on May 31, June 8, and June 16 in the second field. Thrips counts were made on four plants per plot

Table 1.—Comparison of several insecticides applied in dusts for the control of onion thrips on bulb onions in the Coachella Valley, Calif., 1948.

Insecticide	Dosage per Acre	Larvae		Adults		Plants Bolting	Yield in 50-Pound Sacks per Acre
		Per Plant	Reduction	Per plant	Reduction		
	Pounds	*Number*	*Per Cent*	*Number*	*Per Cent*	*Per Cent*	*Number*
Chlordane	5.0	5.4	82	2.1	60	36.0	414
Benzene hexachloride (as gamma isomer)	1.1	5.6	82	2.5	52	35.0	423
DDT	5.3	6.3	80	2.8	46	35.9	386
Lindane	1.1	7.0	77	2.4	54	37.1	432
Toxaphene	10.2	7.7	75	3.1	40	41.6	406
Parathion	1.1	8.4	73	2.7	48	41.0	392
TDE	5.4	10.8	65	3.9	25	28.4	401
Methoxychlor	5.3	15.9	49	3.8	27	27.2	384
Check (no treatment)	—	30.8	—	5.2	—	32.5	355
Difference required for significance (odds 19:1)	—	2.9[1]	—	0.6[1]	—	9.1	[2]

[1] Results from check plots not included in analysis.
[2] Not significant by the *F* test.

at 2 and 7-day intervals after each application, so that six counts were made in each field. Several of the insecticides used in the 1948 experiments were dropped from the 1949 experiment. Benzene hexachloride and lindane were dropped because of possible soil contamination, and methoxychlor because of its ineffectiveness. Aldrin at 1 and 2.5 per cent and difluorodiphenyltrichloroethane, the fluorine analog of DDT, at 10 per cent were included in the 1949 experiments. The diluent for all dusts was western pyrophyllite.

Thrips populations were extremely high in the first field as it was adjacent to a field of earlier onions which were cut and harvested at the time of dusting the experimental field, and this early field furnished a continuous source of reinfesta-

Table 2.—Comparison of several insecticides for the control of onion thrips on bulb onions in the San Gabriel Valley, Calif., 1949.

Insecticidal Dust	Dosage, Pounds per Acre	Larvae per Plant			Adults per Plant			50-Pound Sacks per Acre
		First Field	Second Field	Average	First Field	Second Field	Average	First Field
Toxaphene, 20 per cent	93	20	5.6	13	21	1.2	11	208
Parathion, 2 per cent	94	30	6.3	18	21	1.6	12	222
Aldrin, 2.5 per cent	90	46	6.2	26	19	1.6	10	201
Chlordane, 10 per cent	90	46	6.2	26	30	1.2	16	179
DDT, 10 per cent	94	62	15.7	39	33	1.6	17	179
Aldrin, 1 per cent	96	77	13.5	45	39	1.6	20	136
TDE, 10 per cent	93	79	17.7	48	40	2.2	21	151
DFDT,[1] 10 per cent	91	130	19.9	75	40	2.9	21	158
Check (no treatment)		96	36.5	66	50	4.7	27	129
Difference required for significance (odds 19:1)		21	6.1	40	18	0.6	14	[2]

[1] Difluorodiphenyltrichloroethane, the fluorine analog of DDT.
[2] Not significant by the *F* test.

tion. Infestations were normal in the second field. This discrepancy in populations resulted in a significant interaction between fields when the data were combined, so that few of the differences between treatments were statistically significant. Therefore, data for the two fields are shown individually and in combination, in table 2.

In larval control in the first field a 20 per cent toxaphene dust gave the best results and was significantly better than all other materials except a 2 per cent parathion dust. In the second field dusts containing toxaphene 20 per cent, chlordane 10 per cent, aldrin 2.5 per cent, and parathion 2 per cent were about equal in effectiveness, and were superior to all others. When the data from both fields were combined, the 10 per cent difluorodiphenyltrichloroethane dust was the only inferior one.

In adult control in the first field aldrin at 2.5 per cent, toxaphene at 20 per cent, and parathion at 2 per cent were statistically superior to aldrin at 1 per cent, TDE at 10 per cent, difluorodiphenyltrichloroethane at 10 per cent, and to no treatment. DDT and chlordane at 10 per cent were superior to the untreated plots, and not inferior to the better treatments. In adult control in the second field toxaphene 20 per cent and chlordane 10 per cent were the best treatments and were followed closely by DDT 10 per cent, parathion 2 per cent, and aldrin at 1 per cent and 2.5 per cent. When the data from both fields were combined, the best treatments for adult control were aldrin at 2.5 per cent, toxaphene 20 per cent, and parathion 2 per cent, all of which were statistically superior only to the untreated plots.

Yield records were obtained from the first field. Although no significant differences were apparent, the better treatments (Table 2) gave the highest yields. All the onions in these plots were harvested for yield records.

CONCLUSIONS.—Experiments were conducted in southern California during 1948 and 1949 on the control of the onion thrips on bulb onions with dust mixtures. The results indicate that dusts containing 20 per cent of toxaphene, 2 per cent of parathion, 2.5 per cent of aldrin, 10 per cent of chlordane, benzene hexachloride to give 2 per cent of the gamma isomer, or 2 per cent of lindane may be more effective than a 10 per cent DDT dust. TDE and methoxychlor at 10 per cent were less effective than DDT at 10 per cent.

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Profenusa canadensis, a Pest of English Morello Cherries in Eastern New York[1]

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A cherry or hawthorn sawfly leaf miner was observed as a pest of English Morello cherries near Geneva, N. Y., in 1910 by Parrott & Fulton (1915), and later, in 1937, by the writer in the vicinity of Hudson, N. Y. The insect was identified for Parrott & Fulton by A. D. McGillivray as *Profenusa collaris* McGillivray, but specimens collected in 1939 were identified by R. A. Cushman & G. A. Sandhouse, U. S. Bureau of Entomology and Plant Quarantine, as *P. canadensis* Marlatt.

Observations in English Morello cherry plantings throughout the eastern New York fruit-growing area in 1937 indicated that injury by this leaf miner was confined to a few plantings in the vicinity of Hudson. The number of plantings in which injury can be found and the degree of injury in infested plantings has gradually increased, especially during the last three years. In 1949 injury was general in nearly all plantings of English Morello cherries in Columbia, Dutchess, Orange, and Ulster Counties, and it was not uncommon to find plantings in which 30 to 50 per cent of the leaves were so severely injured by this insect early in June that they could no longer function or had dropped. Fortunately, the English Morello variety constitutes only a small portion of the acreage of sour cherries in the infested area, and the leaf miner prefers it to other varieties even in mixed plantings.

Recent observations indicate that this cherry leaf miner is gradually expanding its host range from hawthorn, its native host, and English Morello cherries to other varieties of cherries. It was first found on the Montmorency variety, the most common variety of sour cherry in New York, in 1946 and has been found in small numbers on several occasions since, particularly in 1948 and 1949. Infested leaves were not found on the sweet cherry variety Windsor until 1949, and then only a few leaves were involved. A Morello cherry called Chase has not been infested even when interplanted with heavily infested English Morello cherries.

The larvae of this cherry leaf miner burrow between the epidermal layers of the leaves and eat out the fleshy area until eventually the leaves appear as large, colorless, blister-like forms. Heavily infested trees appear blighted or burned. Growers unfamiliar with this leaf miner often attribute its injury to spray burn.

This cherry leaf miner has one brood a year and overwinters in the soil in the pupal stage. Emergence records were taken annually near Hudson, N. Y.—from 1938 to 1944 by covering the soil under the trees with cheesecloth cages and from 1945 to 1949 by means of sticky-board traps originally developed for scouting pear psylla infestations (Kaloostian & Yoemans 1944). These records show that adult emergence begins between April 27 and May 12, reaches a peak between April 29 and May 20, and is completed between May 4 and 19. All adults die by May 31. As many as 635 individuals have emerged from the soil under a single tree in an area of 64 square feet. Adults are readily captured on the sticky-board traps, total captures for one trap during the season having been as high as 2001 individuals. Egg laying starts within a few days after emergence. The first eggs have been observed between May 6 and 18 and the first larvae between May 11 and 27. Larvae mature within the leaves rapidly and leave their mines between May 29 and June 3 to hibernate.

Parrott & Fulton (1915) reported that sprays containing naphthalene, kerosene, oil, or nicotine were ineffective in controlling the immature larvae of this leaf miner. Hamilton (1943) reported that a spray containing an aliphatic thiocyanate (25 per cent) 1 pint, powdered cube root (5 per cent rotenone) 4 pounds, and summer oil 4 quarts, in water to make 100 gallons reduced the number of live larvae by 84 per cent. This spray continued to be superior to others in additional tests made from 1943 through 1947, but killed only from 44 to 89 per cent of the larvae.

Tests were conducted in 1945 and 1946

[1] The work reported herein was conducted in cooperation with the New York (Geneva) Agricultural Experiment Station, at Poughkeepsie, N. Y.

with emulsions containing 1 pound of DDT or 1 quart of a 25-per cent DDT emulsion concentrate alone or with 1 gallon of summer oil, per 100 gallons of water. DDT without oil was completely ineffective, but with oil killed between 50 and 75 per cent of the larvae.

In field experiments in 1949 single applications of benzene hexachloride, lindane, or chlordane made on May 17, when larvae were about half-grown, were effective in controlling them. Mortality of the leaf miners was determined by examining 25 injured leaves selected at random for each treatment. The results are given in table 1.

Table 1.—Effect of benzene hexachloride and chlordane sprays on cherry leaf miner larvae, 1949.

INSECTICIDE	QUANTITY PER 100 GALLONS	PER CENT MORTALITY
Benzene hexachloride:		
Wettable powder (6% gamma)	3 lb.	100
	6 lb.	100
Emulsion concentrate (11% gamma)	1 pt.	98.1
	1.5 pt.	100
	1 qt.	100
Lindane, 25% emulsion concentrate	10 oz.	100
	16 oz.	100
Chlordane:		
50% wettable powder	3 lb.	84.8
	6 lb.	100
74% emulsion concentrate	1 pt.	97.6
	1.5 pt.	97.4
	1 qt.	90.9
Untreated		5.9

Tests designed to kill adult leaf miners prior to oviposition were made in large-scale blocks from 1946 through 1949. Two applications were made with DDT each year and with benzene hexachloride in 1949. In 1946 and 1947 the first application was made when the trees were in full bloom, shortly after adults began to emerge, and the second 7 to 8 days later, at petal fall; in 1948 and 1949 the first application was made at the late pink stage of the buds and the second when three-fourths of the petals were off. Results, as shown in table 2, are based on the percentage reduction of leaves injured by this leaf miner as compared with the leaves injured in untreated checks, which averaged 45 per cent in 1946, 12 per cent in 1947, 27 per cent in 1948, and 57 per cent in 1949.

DDT was more effective when the first application was made at full bloom than when made in the late pink stage. However, in New York it is unlawful to apply poisonous sprays at full bloom. In 1947 the set of cherries was reduced 49 per cent when DDT was applied at that stage.

Benzene hexachloride, as used in 1949, was more effective than DDT, and is considered a promising material for use to control cherry leaf miners.

CONCLUSIONS.—*Profenusa canadensis* Marlatt has become a severe pest of English Morello cherries in eastern New York. Although this species seldom infests other varieties, it has occasionally been observed on Montmorency and Windsor varieties.

Table 2.—Results of tests to control the cherry leaf miner by killing the adults, Hudson River Valley, N. Y., 1946–1949.

INSECTICIDE	POUNDS PER 100 GALLONS	YEAR	PER CENT REDUCTION OF INJURED LEAVES
DDT, 50% wettable powder	2	1946	80
		1947	82
		1948	64
	3	1949	9
Benzene hexachloride, wettable powder (6% gamma)	4	1949	88

The most favorable treatments for application at the time most of the eggs are hatched, but while the larvae are still small, are either benzene hexachloride (3 pounds of a wettable powder containing 6 per cent of the gamma isomer, or 1.5 pints of an emulsion concentrate containing 10 to 12 per cent of the gamma isomer, to 100 gallons), or chlordane (6 pounds of a 50 per cent wettable powder, or 1.5 pints of a 74 per cent emulsion concentrate, to 100 gallons). Benzene hexachoride is a little more effective than chlordane. The adult population can be materially reduced and egg laying curtailed markedly by making two applications of benzene hexachloride (4 pounds of wettable powder containing 6 per cent of the gamma isomer, to 100 gallons), the first at the time buds are pink and the second at petal fall. Two applications of DDT will reduce the adult population materially, but this treatment cannot be recommended since the first application must be made while the trees are in bloom and may reduce the set of fruit seriously.

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Tests with DDT to Control Black Fly Larvae in Alaskan Streams[1]

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Tests made during 1947–48 in Alaska indicated that black fly larvae can be controlled easily with very light dosages of DDT applied to streams with hand or airplane equipment (Gjullin *et al.* 1949). Therefore, in 1949 these methods of controlling black flies (*Simulium* spp.) were tested further in the vicinity of Naknek, Alaska, where all streams were treated early in the season with a DDT airplane spray in an effort to eliminate overwintering larvae. The results of these tests were so unsatisfactory that small-scale tests were made in Otter Creek, near Anchorage, to determine why the treatments at Naknek were ineffective.

AIRPLANE TESTS.—For all the airplane tests a C-47 airplane was used to apply a solution containing 20 per cent of DDT and other ingredients.[4] The plan was to spray streams when they were free from ice in the spring and to repeat the sprays during the season when inspection showed that larvae were present. When the area was first inspected on April 25, the Little King Salmon Creek was frozen on the bottom and the rocks could not be inspected for black fly larvae. A wide ice ledge on the Naknek River also prevented examination of this stream. These streams and Pauls Creek were free of ice when examined again on May 5. An average of five full-grown larvae per rock was found in the Little King Salmon Creek; in the Naknek River there were few large larvae and occasional pupae, but small larvae were present on favorably situated rocks in numbers of 100 or more. The Naknek River had been partially open for several weeks and these larvae may have been from a spring hatch of overwintering eggs. Few rocks suitable for attachment of larvae were found in Pauls Creek, but larvae were numerous on vegetation.

On May 5 two swaths about a mile apart were sprayed across the Little King Salmon Creek. The applications were made at the rate of 0.1 pound of DDT per acre based on a swath width of 800 feet. No reduction in the numbers of larvae was noted in 24 hours and an additional swath was sprayed across the stream. This application also caused no reduction of the larvae on rocks 300 feet downstream. These results were in marked contrast to those obtained in July and August of the previous year, when complete detachment occurred from 1.5 to 2.5 miles downstream after similar applications.

On May 7 the Naknek River was sprayed from the Naknek Air Base to Naknek Lake. All other streams emptying into the Naknek River were sprayed for a distance of about 30 miles upstream or to their point of origin. The spray was applied on 800- to 1000-foot sections of each stream at intervals of about 1 mile. Treatments were made either in a single swath across streams or by turning the spray on for 8 to 10 seconds while flying upstream. Twenty-four hours later all the small larvae had detached and only about one large larva per rock remained in the Naknek River. In the Little King Salmon Creek there was no reduction in the number of larvae.

On May 17 only two pupae were found after the examination of many rocks in the Naknek River. The Little King Sal-

[1] This work was conducted under funds allotted by the Department of Defense to the Bureau of Entomology and Plant Quarantine.

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[4] 40 per cent of *Velsicol AR-50* (chiefly mono- and dimethylnaphthalenes), and 40 per cent of fuel oil.

mon Creek could not be checked for larvae because of high waters. No larvae were found in Little King Salmon Creek or the Naknek River when they were examined again on June 1.

On June 25 the Naknek River and Little King Salmon Creek were checked and from 50 to more than 100 larvae were present on favorably situated rocks. Larvae were abundant also on trailing vegetation and sticks. They were of all sizes and an occasional pupa was seen in the Naknek River. All streams were again sprayed in the same manner as on May 7. Because of winds ranging from 12 to 24 miles per hour, the spray was delivered from a pipe under one wing, rather than through the booms. The large droplets came down well in the wind and the streams appeared to be covered even better than when the booms were used. Nearly complete control resulted in 4 hours.

SMALL-SCALE TESTS.—In the small-scale tests an average of about five full-grown larvae per rock was present when the upper portion of Otter Creek first became ice-free in May. Applications of the 20 per cent DDT airplane spray and of an emulsion concentrate[1] were made during the third week in May. The materials were sprayed on the surface of the water with a small hand sprayer over a period of 15 minutes.

The emulsion at a concentration of 0.2 p.p.m. of DDT caused 30 per cent of the larvae to detach in 24 hours, and at 0.4 p.p.m., only 8 per cent. The 20 per cent airplane spray at rates of 0.3, 0.5, and 2 p.p.m. caused 22, 29, and 60 per cent detachment, respectively, in 24 hours. The water temperatures for these three tests were 42°, 50°, and 62° F. At similar temperatures, during July and August in 1947 and 1948, 0.4 p.p.m. of DDT always caused complete detachment of larvae within 24 hours in this and other streams.

The seasonal difference in the effectiveness of the same treatments indicates that the overwintering larvae are less susceptible to DDT than new-generation larvae and that considerable difficulty may be experienced in controlling them by early-spring treatments. Further tests are necessary to determine whether late-fall treatments will be effective against potential overwintering larvae.

SUMMARY.—Tests during the summers of 1947 and '48 indicated that new-generation black fly larvae could be controlled easily with light dosages of DDT applied to streams with hand or airplane equipment. Early in May 1949 sprays containing 20 per cent of DDT and other ingredients[2] were applied by airplane to the Naknek River and its tributaries near Naknek, Alaska, at a rate of 0.1 pound of DDT per acre, to determine the effect on the overwintering black fly larval population. The treatments caused detachment of small larvae but had little or no effect on the large overwintering larvae. Similar treatments to the same streams in late June gave complete control of all stages of new-generation larvae.

Hand applications made in the third week of May at 0.3, 0.5, and 2 p.p.m. for 15 minutes caused only 22, 29, and 60 per cent detachment of overwintering larvae in a stream, whereas at similar temperatures (42°, 50°, and 62° F.) in July and August of previous seasons, 0.4 p.p.m. was completely effective against new-generation larvae.

The seasonal difference in the effectiveness of the same treatments indicates that overwintering black fly larvae are less susceptible to DDT than new-generation larvae and tests are needed to determine whether late-fall may not be the optimum time for treatments to clear streams of potential overwintering populations.

[1] 20 per cent of DDT, 70 per cent of *Velsicol AR-50*, and 10 per cent of *Triton X-100* (monoisooctyl phenyl polyethylene glycol ether).

[2] 40 per cent of *Velsicol AR-50* (chiefly mono- and dimethylnaphthalenes), and 40 per cent of fuel oil.

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Field Tests on Control of American Dog Tick in Massachusetts[1]

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The American dog tick, *Dermacentor variabilis* (Say), is extremely abundant in many sections of the New England States. Besides being a source of annoyance to man and animals, it is a vector of Rocky Mountain spotted fever and tularemia. Smith & Gouck (1945), Bishopp *et al.* (1946), and Gouck and Smith (1947) have reported that this species can be controlled by ground applications of 1 to 3 pounds of DDT per acre, in a dust, emulsion, or oil solution. Glasgow & Collins (1946, 1948) reported good control with 1 pound of DDT per acre applied in oil solution with both ground equipment and airplanes.

Studies were initiated by the senior author in southeastern Massachusetts in 1948, and continued by the junior author in 1949, to compare the effectiveness of DDT and several other insecticides against this species. Most of the tests were made on small roadside plots, several types of ground equipment being used to apply the treatments. Additional tests were made with DDT applied by helicopter and airplanes. In 1949 emphasis was placed on the effect on ticks of airplane applications of DDT to large areas for the control of the gypsy moth, *Porthetria dispar* (L.). The results of all these studies are presented in this paper.

SMALL-PLOT TESTS. *Emulsions.*—Small-plot tests were conducted during the summer of 1948 at Martha's Vineyard with emulsions of DDT, toxaphene, chlordane, and parathion. The plots were narrow strips (1000 by 21 feet) along roadsides heavily infested with dog ticks. Duplicate plots were treated with parathion at 0.5 pound per acre and with the other insecticides at 1.0 and 2.5 pounds per acre. These dosages were applied as emulsions at a rate of 15 gallons per acre, with a small power sprayer. The 2.5-pound treatments were made on May 28–29 and the other treatments on June 9–10.

Tick counts were made before and at intervals after treatment by means of cloth drags, several counts being made in each plot. Two untreated plots were established as checks. Because of extreme variations in tick abundance between these two plots, the percentage of control in the treated plots is based on the original counts only and is not adjusted to compensate for changes in the check plots. The results of these tests are given in table 1.

DDT was the most effective of the materials, giving an average of better than 90 per cent reduction of ticks over a 6-week period with both the 2.5- and 1-pound dosages. The plots treated at the higher dosage still showed about 90 per cent control at the end of 8 weeks. With toxaphene and chlordane the 6-week averages ranged from 83 to 88 per cent for the 2.5-pound dosages, and from 63 to 88 per cent with the 1-pound treatments. Parathion at 0.5 pound per acre was relatively ineffective after the first week.

Oil Solutions.—Oil solutions were used to treat two roadside plots with DDT and one with toxaphene in 1948, and one plot each with DDT, toxaphene, chlordane, and dieldrin in 1949. Each insecticide was applied as a 12 per cent oil solution at the rate of 1 pound of active ingredient per acre in 1 gallon of oil. In 1948 the solutions were applied with a small mist blower, and in 1949 with a large mist sprayer. Tick counts were made by dragging a cloth along the ground before and at weekly intervals after spraying. The results of these tests are given in table 2.

In 1948 DDT and toxaphene gave about the same results over a 4-week period, both averaging better than 90 per cent control. The dosage used in 1 gallon of oil gave nearly as good control of ticks as the same dosage applied in 15 gallons of emulsion (Table 1).

In 1949 DDT and dieldrin gave almost complete control over a 5-week period and were clearly superior to toxaphene and chlordane. Neither toxaphene nor chlordane was highly effective the first week after treatment, but both showed increasing effectiveness subsequently,

[1] This work was conducted under funds allotted by the Department of Defense to the Bureau of Entomology and Plant Quarantine.

[2] Albert N. Davis assisted in making these tests.

Table 1.—Control of adult American dog ticks in roadside plots, Martha's Vineyard, Mass., 1948. Average of 2 replications.

Insecticide	Pounds per Acre	Pre-Treatment Count	Per Cent Reduction or Increase at Indicated Weeks After Treatment								
			1	2	3	4	5	6	7	8	Average for 6 Weeks
DDT	2.5	340	99	99	96	91	98	85	88	90	94.7
	1	249	93	87	92	93	93	94	—	—	92.0
Toxaphene	2.5	308	87	94	80	81	98	82	82	87	87.0
	1	202	57	69	81	80	75	80	—	—	73.7
Chlordane	2.5	131	95	90	85	82	94	67	77	74	85.5
	1	268	72	75	90	87	84	91	—	—	83.2
Parathion	.5	224	94	74	75	67	72	63	—	—	74.2
Untreated checks:											
For 2.5-pound treatments	—	314	+4	20	+56	+32	27	62	64	85	3
For other treatments	—	160	+20	+146	+76	+326	+409	+388	—	—	+227

probably attributable in part to the seasonal decline in the tick population.

AERIAL SPRAYING OF DDT-OIL SOLUTIONS.—In 1948 observations were made over a period of 5 weeks on an area that had been treated by helicopter at the rate of 1 pound of DDT per acre (1 gallon of 12 per cent DDT-oil solution). The area observed was 125 feet wide and 1450 feet long, and was bisected by a road, along the sides of which were heavy infestations of ticks. The area had been treated from a height of 20 feet in a single flight down the road, or center of the area. A similar untreated area was used as a check. Fourteen to 18 drags of 150 feet each were made in the treated and untreated areas at each observation, and the percentage control was adjusted by Abbott's formula for the increase or decrease of populations in the check plot.

Counts made 3 days after spraying showed a 94 per cent reduction of ticks in the treated area and equally effective control was indicated after 4 weeks. However, after 37 days a control of only 80 per cent was indicated.

Observations were made during May and June 1949 in the Cape Cod area to determine the effect of gypsy moth control operations on the abundance of the American dog tick. The control program involved the aerial treatment with DDT of approximately 200,000 acres, the area being divided arbitrarily into a number of sections to facilitate spraying. The planned rate of application was 0.5 pound of DDT per acre, but computations from daily flight records and the amount of solution used showed that the dosages varied from 0.25 to 1.0 pound per acre. Several sections were intentionally treated

Table 2.—Comparative toxicity of four compounds against adult American dog ticks, 1948 and 1949. (Materials applied as 12 per cent oil solutions at a rate of 1 pound per acre.)

Insecticide	Pre-Treatment Counts	Per Cent Reduction at Indicated Interval After Treatment						
		1–2 Days	1 Week	2 Weeks	3 Weeks	4 Weeks	5 Weeks	4- or 5-Week Average
			Tests in 1948					
DDT	270	—	95	96	94	95	—	95.0
	196	—	87	93	83	90	—	88.3
Toxaphene	130	—	99	93	91	88	—	92.8
Untreated check	85	—	22	7	13	32	—	18.5
			Tests in 1949					
Dieldrin	211	91	99+	100	99+	99+	96	98.6
Toxaphene	99	50	75	97	88	93	95	89.6
Chlordane	180	32	65	87	78	94	94	83.6
DDT	86	99	100	100	99	100	99	99.6
Untreated check	63[1]	—	—	—	+160	11	13	—

[1] The first counts in this plot were made the second week of the tests.

Table 3.—Relative abundance of adult American dog ticks in the Cape Cod area before treatment, 1949.

Location	Average Number of Ticks per 150 Feet of Drag								
	May 6–12	May 13–19	May 20–26	May 27–June 2	June 3–9	June 10–15	June 16–23	June 24–30	July 1–7
Harbor Bluff	20.6	15.9	Treated May 22						
Sippowissett	12.0	13.8	4.2	Treated May 30					
Spring Hill Rd.	22.2	—	18.9	Treated May 29					
Falmouth	38.6	24.8	60.0	Treated May 29					
East Brewster	—	94.3	—	210.8	Treated June 3				
East Dennis	—	68.2	—	132.5	—	Treated June 10			
Pocasset	26.1	21.6	—	—	9.2	Treated June 6			
Handy Point	—	10.7	—	—	44.6	Treated June 6			
Dennis	—	19.2	—	—	62.4	Treated June 10			
Wings Island	—	9.3	—	—	26.9	Treated June 12			
Pochet Neck	—	13.7	—	—	34.7	34.1	35.3	Treated June 17	
Cedarville	—	—	—	—	—	6.3	10.2	5.6	5.5

with 1.0-pound dosages for comparative purposes. As usual, these dosages were the amount dispersed, and little information was obtained as to the amount that actually reached the ground. In a treatment at East Brewster plates exposed on the ground showed a deposit of only about 10 per cent of the amount dispersed. The sprays were applied with several types of aircraft and aerial spray equipment, and differences in performance were probably reflected, to some extent at least, in the dosage variations and subsequent differences in the effectiveness of treatments.

Counts of ticks on cloth drags were made at 21 representative locations before and at weekly intervals after spraying. Ten drags of 150 feet each along road-

Table 4.—Effects of aerial DDT spraying on populations of the American dog tick at Cape Cod, 1949.

Location	Date of Treatment	Dosage of DDT (Pounds per Acre)	Pretreatment Counts	Per Cent Reduction at Indicated Time After Treatment			
				1 Week	2 Weeks	3 Weeks	4 Weeks
Spring Hill Road	May 29	1.0	190	83.2	91.6	71.0	85.3
East Brewster	June 3	1	1,265	87.4	79.0	79.1	96.8
East Dennis	10	1	795	80.1	75.6	95.5	—
Dennis	10	1	624	90.1	94.9	97.6	—
Average			718.5	85.2	85.3	85.8	91.1
Yarmouth Station	May 16	.5	80	50.0	—	—	95.0
Yarmouth Port	17	.5	72	37.5	—	—	79.2
Hyannis Port	17	.5	168	0	—	—	20.2
Sagamore	27	.5	68	19.1	83.8	75.7	71.6
Falmouth	29	.5	900	12.6	64.0	53.6	9.8
Average			257.6	23.8	73.9	64.7	55.2
Camp Edwards	June 2	.4	98	73.5	68.4	65.3	93.9
Handy Point	6	.4	446	80.7	34.3	56.9	84.5
Wings Island (1949)	12	.4	431	50.6	81.7	93.0	—
Wings Island (1948)	12	.4	170	82.4	89.4	94.7	—
Pochet Neck	17	.4	494	95.7	98.4	—	—
Average			327.8	76.6	74.4	77.5	89.2
West Barnstable	May 22	.3	280	53.6	—	72.5	45.0
Harbor Bluff	22	.3	106	91.5	—	87.7	53.8
Average			193	72.6	—	80.1	49.4
Yarmouth	17	.25	596	9.7	—	—	84.7
Sippowissett	30	.25	35	0	48.6	0	22.9
Average			315.5	4.8	—	—	53.8

sides and paths were usually made at each location. As suitable untreated areas were not available for comparison, the effectiveness of the treatments was determined solely on the difference in counts before and after treatment. As shown in table 3, however, counts made in a number of sections up to the time of treatment indicated that the general tick populations remained fairly constant through June.

The effects of the treatments in the various sections are shown in table 4. Applications of 1 pound of DDT per acre in four areas caused an average reduction of ticks of about 85 to 91 per cent the first 4 weeks after treatment. The average reduction over a period of 3 to 4 weeks ranged from 82.8 to 94.2 per cent in the different areas. Treatments at lower dosages, 0.25 to 0.5 pound per acre, were generally much less effective than the 1-pound dosage. These results indicate that DDT aerial sprays, to be highly effective under the conditions existing in these areas, must be applied at dosages greater than 1 pound per acre.

SUMMARY.—Field tests were conducted in southeastern Massachusetts in 1948 and 1949 to compare the effectiveness of DDT and several other insecticides for the control of the American dog tick, *Dermacentor variabilis* (Say). Extensive observations were also made to determine the effectiveness against this tick of airplane applications of DDT to large areas for the control of the gypsy moth, *Porthetria dispar* (L.).

In comparative tests on small plots, DDT and dieldrin were the most effective materials, 1 pound per acre of each showing over 90 per cent control of ticks for 5 to 6 weeks. DDT at 2.5 pounds per acre gave effective control for at least 8 weeks. Toxaphene and chlordane were slightly less effective than DDT at both 1- and 2.5-pound dosages. Parathion at 0.5 pound per acre was relatively ineffective after the first week. Applications of 1 pound per acre of DDT, toxaphene, and chlordane in 1 gallon of oil (12 per cent solutions) gave about as good control as the same dosage applied in 15 gallons of emulsion.

Observations in areas treated by airplane showed 85 to 91 per cent control of ticks for 4 weeks, with a dosage of 1 pound of DDT per acre. Applications of 0.25 to 0.5 pound per acre varied considerably in effectiveness but were generally much less effective than the 1-pound dosage. A 1-pound-per-acre application with a helicopter operating under optimum conditions gave about as good results as this dosage applied on small plots with ground equipment.

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M. A. YOTHERS RETIRES

M. A. Yothers, of Yakima, Washington, retired from active service in the Bureau of Entomology and Plant Quarantine on July 1, 1949, after a period of 32 years service. His retirement was preceded by leave of absence for a year.

Since March, 1949, Mr. Yothers has been serving as Entomologist of the Washington State Department of Agriculture as Entomologist and has been conducting studies on the oriental fruit moth in the Yakima region. To these studies will be added work on the cherry fruit fly during the present year.

Mr. Yothers has been engaged in entomological work at various locations for a period of 42 years in Washington, Oregon and Michigan.

Observations on the Control and Ecology of Black Flies in Pennsylvania[1]

ROBERT L. GOULDING, JR., and C. C. DEONIER,[2] *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*

Studies on the control of black flies (Simuliidae) over large areas were made during the spring and summer of 1948 in the Pocono Mountains near Wilkes-Barre and Scranton, Pa., where a large-scale DDT spray program against the gypsy moth was being conducted by the Bureau of Entomology and Plant Quarantine. Two test areas having streams favorable for the development of black fly larvae were selected for observation. Area I, in Madison and Covington townships, Lackawanna County, contained 18,500 acres and area II, in Bear Creek, Buck, and Clifton townships of Luzerne and Lackawanna Counties, contained 48,250 acres. Area II was bounded on one side by the Lehigh River and included almost all the Bear Creek watershed. An adjacent untreated check area with streams similar to those in the treated areas was selected in Monroe County.

METHOD OF TREATMENT.—An airplane spray containing 12 per cent of DDT in kerosene-xylene was applied at the rate of 1 pound of DDT per acre. The applications were made with three light biplanes and one C-47. Open bodies of water and large streams were avoided, or treated only lightly, to prevent possible damage to fish. As a result many of the areas with high black fly populations received considerably less than 1 pound of DDT per acre.

Different parts of area I were sprayed on May 1, June 12, 16, and 17, but in June field and truck crops had developed to such an extent that it was considered inadvisable to cover the cultivated area. Since only the wood lots, roadside hedges, and fields containing shrubs were sprayed, many of the streams were not exposed to direct spray along their entire length. Spraying in area II was begun on May 10 and completed on June 14. This area was given a complete coverage of DDT except for the lakes and ponds.

SAMPLING METHODS.—Observation stations were established along the streams to cover as much of each area as possible. The effectiveness of the treatment was determined by making counts of larvae, pupae, and adults at each station at intervals before and after the spraying. Counts of the immature stages were averages of the numbers of larvae and pupae per 5 or 10 attachment units, which consisted of individual rocks 3 to 10 inches across, single blades of grass 6 inches long, or leaves 2 to 5 inches across. In dense populations the number of larvae per square inch or per square foot of attachment surface was counted or estimated. In all cases only the most favorably situated units were used in the sample.

Samples of adults were taken by sweeping with a net in front of the observer. When populations averaged less than one adult per sweep, additional counts were made of the adults passing between the observer and some distant object during a period of several minutes. Attempts to collect resting adults with a sweep net were unsuccessful. Where adults were not observed in flight, none was taken in sweeping brush and grass. Adult biting was so sporadic that estimates of the population on this basis were unreliable.

RESULTS. *Control of Larvae.*—In area I pretreatment larval counts at 8 stations in 6 streams ranged from less than 1 to 9 larvae per unit. The larvae were completely eradicated, and the streams were still free of larvae 2 weeks after the spraying.

In area II pretreatment counts at 27 stations in 10 streams ranged from less than 1 larva per rock to 200 per square inch. Five artificial lakes, all giving rise to streams, are situated in the area. Some of the heaviest breeding occurred just below three of these lakes. Some pupation and adult emergence was noted before the spraying was completed on June 14.

The larvae in area II were apparently eradicated except at one station where an average of 1.1 larvae per unit was ob-

[1] This work was conducted under funds allotted by the Department of Defense to the Bureau of Entomology and Plant Quarantine.

[2] Appreciation is expressed to J. M. Corliss, in charge, and to K. A. Haines, W. H. Hanley, and their associates, Division of Gypsy and Brown Tail Moths Control, Bureau of Entomology and Plant Quarantine, for their interested cooperation in this study, and to F. R. Regan for assistance in making the tests.

tained 60 days after the spraying (compared with a pretreatment count of 169 per square inch). This station was situated on a narrow strip of land separating two artificial lakes, and probably received only a very light treatment from drifting spray.

The spray applications evidently did not leave an effective DDT residue, because larvae taken from an untreated stream survived for 1 week when placed at a treated station immediately after the oil on the water had disappeared. The original population at this station had disappeared completely within 2 hours after the treatment.

The treatment of 0.25 mile of the headwaters of a stream in area II caused detachment of larvae 0.75 mile below the area of direct treatment (Table 1), although the amount of DDT actually striking the water was far less than 1 pound per acre. The time required for complete detachment of larvae was 20 hours, as compared with the usual 3 hours or less.

Table 1.—Effects of DDT airplane sprays on black fly larvae at different distances downstream from point of application. Larvae were attached to rocks.

Miles from Treatment	Pre Treatment Count	Number of Larvae at Indicated Time After Treatment		
		5 Hours	12 Hours	20 Hours
0	200	5	1	0
—	100	8	3	0
.25	44	1	0	0
.75	23	—	—	0
1.25	37	—	—	2
1.75	20	—	—	5
2.25	18	—	—	6
2.75	27	—	—	28

A net was placed across the stream at the 0.25-mile station to catch the detached larvae. Five hours after the spray application the net was slimy with detached larvae, 20 per cent of which were still alive. After 20 hours all the larvae apparently were dead. Larvae in an untreated stream were dislodged by hand in great quantities into a net. When observed on the following day, less than 20 per cent of the larvae remained on the net, but all were normal.

Six lake-fed streams in the check area were observed, as well as other streams similar to those found in the treated areas. Breeding continued in these streams throughout the study, although instances of natural decrease in the larval population were noted. Such decreases were attributed to the encroachment of filamentous algae on attachment surfaces and the deposition of silt following floods.

Control of Pupae.—In four sections of streams having heavy pupal populations the spraying had no apparent effects on this stage. Adults emerged within 6 to 7 days, after which only empty pupal cases were found.

Control of Adults.—Some detailed studies were made on adult activity in the check areas and in treated area II. Adults were observed in the treated area only during emergence and in the immediate vicinity of their place of emergence. It appeared that sufficient DDT residue was present on emergent vegetation or stream banks to control adults that emerged from pupae present at the time of spraying.

On the final survey during the second week of July, adults were not observed at any of 27 stations in the treated area, but were active at 8 of the 30 stations in the check area. Oviposition was noted at 7 stations in the check area.

REINFESTATION OF STREAMS.—During April a survey was made of some of the streams in areas that had been similarly treated in 1946 and 1947. These areas adjoined those under observation in 1948 and contained streams that appeared to be equally favorable for black fly breeding. Larvae were found only in streams in the Rattlesnake Creek watershed.

In 1948, with the exception of one station in the Bear Creek area previously mentioned, no reinfestations of streams were noted. During May in 1949, a brief check of some of the treated areas was made by C. C. Deonier, and breeding was found in Meadowbrook Run in the center of the Bear Creek area. Breeding was also noted in Ash Creek and Buckeye Run, both of which were sprayed in 1947 and were free of larvae in 1948.

ECOLOGICAL OBSERVATIONS.—Breeding was observed in stream types varying from a mountain cascade with a flow rate greater than 4 feet per second to sluggish streams in swamps with flow rates of less than 1 foot per second. Larvae were attached to stones, wood, leaves, grass blades, glass, trash, and in one case on a

recently skinned deer hide. The largest larval populations occurred immediately below the outlets of lakes, ponds, and bogs. Heavy breeding was also observed in pasture streams.

Eggs were found at or just below the water surface on grass, leaves, and waterlogged paper, but rarely on rocks. Although oviposition was heaviest where adults were most abundant, some eggs were found in locations completely free of larvae and pupae.

The three predominating species[3] of black flies in the areas under observation were *Simulium venustum* Say, *S. vittatum* Zett., and *Cnephia dacotense* (D. and S.). Also found were *Prosimulium hirtipes* (Fries), *P. magnum* D. and S., *S. decorum* Walk., and *S. costatum* Fries. Where large numbers of larvae occurred in a breeding area, mixed populations were the rule.

SUMMARY.—Observations were made in streams in several large areas near Wilkes-Barre and Scranton, Pa., to determine the effect on black fly populations of DDT sprays applied by airplane for the control of the gypsy moth. A 12 per cent solution of DDT in kerosene-xylene was applied at the rate of 1 pound of DDT per acre over most of the area. Large streams and open bodies of water were avoided, or treated only lightly, to prevent possible damage to fish.

Complete control of black fly larvae was obtained at 34 stations in 15 streams out of a total of 35 stations in 16 streams.

Pupae apparently were not affected by the spray. Adults were present in the breeding area immediately after the spraying but were not observed 1 week afterwards, although many had emerged from the pupae present at the time of spraying. Oviposition was not observed in the treated areas. It is inferred that sufficient DDT residue existed to control the emerging adult population.

Simulium venustum Say, *S. vittatum* Zett., and *Cnephia dacotense* (D. and S.) were the predominating species. Small numbers of *Prosimulium hirtipes* (Fries), *P. magnum* D. and S., *S. decorum* Walk., and *S. costatum* Fries were also found. The more dense larval populations were found immediately below the outlets of lakes, ponds, and bogs.

[3] Identification of selected specimens was made by Alan Stone of the U. S. National Bureau.

Entomogenous Fungi of the Family *Entomophthoraceae* in the Pacific Northwest

L. P. ROCKWOOD,[1] *Bureau of Entomology and Plant Quarantine, Agr. Res. Adm., U.S.D.A.*

For a number of years the writer made observations on entomogenous fungi, especially during the period 1932 to 1941, when facilities were available at the Forest Grove, Oregon, station of the Bureau of Entomology and Plant Quarantine.

The entomopthorous fungi are undoubtedly the most effective agents of fungous disease among insects. Under favorable conditions epizootics reduce large and destructive outbreaks of some insects to negligible proportions. The writer has often observed such phenomena in the Willamette Valley of Oregon. In that area the larvae of the clover leaf weevil, *Hypera punctata* (F.), are every year prevented from doing appreciable injury to the clovers and alfalfa by *Empusa* (*Entomophthora*) *sphaerosperma* (Fres.) Thaxt., whereas in some of the more arid areas east of the Cascade Mts., appreciable damage is often done to red, alsike, and Ladino clovers, and more rarely to alfalfa by this weevil.

A species of fungus on aphids, which agrees in all particulars with Thaxter's description of *Empusa aphidis* Hoffman (1888), has several times reduced to negligible numbers enormous populations of the pea aphid, *Macrosiphum pisi* (Ktlb.), on common vetch and on Austrian winter field peas, and the plants have recovered from severe aphid injury. This fungus is so omnipresent in the area that results of experiments with chemical control cannot be easily appraised and may be misleading if the incidence of the fungous disease is not taken into consideration.

[1] Retired October 31, 1948.

Another species of fungus on aphids, which agrees with Thaxter's (1888) description of "*Empusa planchoniana* (Cornu)?" and which Petch (1937) has named *E. thaxteriana*, also occurs in Oregon. Miss Vera K. Charles' (1941) record for Oregon was based on collections made in the Williamette Valley. In early spring, even as early as February, colonies of pea aphids showed a combined infection by *E. aphidis* and *E. thaxteriana*, but *E. aphidis* predominated and only a few spores of *E. thaxteriana* were produced. *E. aphidis* fastens its victims to leaves by rhizoids, usually near the tops of the plants, but *E. thaxteriana* does not produce rhizoids, in consequence of which its victims fall to the ground. The importance of *E. thaxteriana* may be underestimated when both species are present, but the writer believes that *E. aphidis* is the most effective fungus on the pea aphid in the Williamette Valley. In more arid regions, where the annual average precipitation is about 10 inches, *E. thaxteriana* appeared to replace *E. aphidis*. In the Palouse area of Washington and Oregon, where the average precipitation is about 25 inches, *E. aphidis* and *E. thaxteriana* occurred in nearly equal abundance.

The fact that aphids killed by *Empusa thaxteriana* fall to the ground, where conditions are more humid in irrigated fields than on the tops of the plants where victims of *E. aphidis* are found, suggests that *E. thaxteriana* is better adapted to survive in the semiarid regions where the night humidity is often low. Moreover, resting spores are known to occur in *E. thaxteriana*, but none were certainly noted in *E. aphidis*. In any case, neither species seemed to be effective enough to act as a serious check to pea aphid populations east of the Cascade Mts. in the Pacific Northwest.

Another fungus, which agrees with Thaxter's (1888) description of *Empusa fresenii* Now., has occasionally been found on aphids in the Willamette Valley in July, notably on *Macrosiphum pisi* on Scotch broom near the Molalla River and on *M. granarium* (Kirby), *Aphis rhopalosiphum prunifoliae* (Fitch), and *Myzus persicae* (Sulz.) on oats near Forest Grove.

Caterpillars, particularly noctuid larvae, are frequently killed by a species of fungus, which Thaxter (letter of February 28, 1926) has determined to be *Empusa virescens* Thaxt. This species killed many larvae of *Dargida procincta* (Grote) that occurred in great numbers on canary grass meadows on the tidelands near Coquille, Oregon, in 1928. An outbreak of the army cutworm, *Chorizagrotis auxiliaris* (Grote), that occurred near Washtucna, Adams County, Washington, in the fall of 1926 had denuded several hundred acres of rye and wheat by November, when the cut worms were still in the early stages. It was feared that a destructive outbreak was in prospect for the spring of 1927. However, when the area was visited on March 15, 1927, by the late Anthony Spuler of Washington State College and the writer, only dead larvae were found in the grain fields. These dead larvae contained resting spores of an entomophthorous fungus. The dimensions of the spores (40 microns in diameter) and of an old dead conidium (30 microns by 12 microns) indicated that this fungus might have been *E. virescens*. Many healthy larvae of this cutworm were found in their natural habitat, grassy coulees, while at the same time only dead ones were found in the grain fields.

Grasshoppers, *Melanoplus femur-rubrum* (DéGeer) and *M. mexicanus* (Sauss.), have been found dead on the tops of alfalfa plants in considerable numbers on a few occasions in irrigated fields east of the Cascade Mts. The dead grasshoppers contained resting spores that agreed with descriptions of *Empusa grylli* (Fres.) Now. The writer, in common with other workers, was unable to induce these resting spores to germinate, even after they had overwintered.

Empusa muscae Cohn often kills diptera in the fields of western Oregon. Syrphid flies and dung flies (Scatophagidae) have been killed by it in considerable numbers at various times.

A fungus species, probably *Empusa forficulae* (Giard) Petch, is frequently found on European earwigs, *Forficula auricularia* L., in western Oregon and is believed to be an important factor in the natural control of that pest.

Most of the writer's studies in this group of fungi were on *Empusa aphidis* as a natural enemy of the pea aphid on fall-sown annual legumes. It was noted that aphids killed by this fungus first became abundant in fields of vetches or Austrian

winter field peas that had become infested by pea aphids in the preceding fall. The fungus became established in such fields at about the same time as the aphids and persisted over winter. In such fields the aphid populations were usually checked by fungous disease in April. In other fields that had been seeded late enough to escape fall aphid infestation, both the aphid abundance and the fungous disease built up a month or more later.

In cages the fungus did not develop when the original, parent aphids were free of disease at the time the cages were started. Hence the only means of the dispersion of the fungus appears to be largely by infected alate aphids, which were impelled to migrate in an early stage of infection. Similarly, apterous aphids became restless in the early stages of infection and moved to the topmost leaves of their host plants in the late afternoon. The aphids died in the early evening and were fastened by the fungous rhizoids to the leaves where they had died. If the relative humidity attained 90 per cent or more in the night, the spore-bearing hyphae emerged through the skin of the aphid and spores were discharged. If the pale swollen aphids were collected in the late afternoon shortly before or after death and were placed in dry vials at relative humidities below 60 per cent, the hyphae did not emerge. Such mummified aphids could be kept indefinitely and did not shrivel on drying out.

The writer performed an experiment in which these mummified aphids were transferred to slides in a moist chamber at intervals of a few days. The dead aphids rapidly absorbed moisture from the air, and if the fungus was still alive, spores were projected during the following night. By these means it was discovered that the fungus could survive, in the aphids, under dry conditions for 3 weeks, and in 42 per cent of a sample for 4 weeks. These experiments indicate how this fungus could persist over dry periods in the fields, even though it is not known to produce resting spores. They also explain how the spread of the fungous disease is stopped during periods when the night humidity does not attain 90 per cent.

Empusa aphidis can be separated from *E. thaxteriana* in the aphid, before spore production, by the characteristics of the hyphae. The hyphae of *E. aphidis* are long and comparatively slender and in the later stages show well-defined nuclei, only one of which goes into each conidium. The hyphae of *E. thaxteriana* are short, stout, and contorted, and the nuclei are concealed in the granular protoplasmic contents. The conidia of *E. thaxteriana* are projected farther than are those of *E. aphidis*. The large spherical resting spores of *E. thaxteriana* were often observed in process of development in the aphids.

It was thought for many years that the Entomophthoraceae were obligate parasites and could not be cultured. Of late years Speare (1912) and especially Sawyer (1929) have demonstrated that artificial culture of these fungi is possible. In March 1934 the writer succeeded in getting pure cultures of *E. aphidis* from the pea aphid. This was accomplished by the following means: Collecting material consisted of sterilized small dry glass vials plugged with cotton and a camel's hair brush sterilized by dipping in 90 per cent alcohol before each collection. Individual aphids that were pale and swollen and located on the tips of vetch were collected in the later afternoon. These specimens, in their individual vials, were brought into the laboratory. and each aphid was dropped from the collecting vial onto a previously prepared disk of sterilized potato in a small petri dish. The dishes were then covered and allowed to stand overnight at room temperatures. By the following day, or sometimes after another night, the fungus had developed profusely on the specimens. Transfers, at first to egg medium but later direct to potato, were made, most successfully by picking off bits of sporulating hyphae with a sterilized needle. A fair percentage of these transfers would be free of contamination by other fungi or bacteria long enough to get cultures that would be pure when transferred.

The fungus grew readily on egg-yolk-milk, whole egg-milk, egg yolk, smelt, salmon, pork, veal, beef, potato, and carrot. Cultures on halibut and on parsnip appeared to be abnormal. Egg, fish, and meat were liqueified by the proteolytic action of the fungus after 3 to 4 months at temperatures around 50° F. Cultures remained viable for 3 months at this temperature in the winter months, and for 4 months in an electric refrigerator at tem-

peratures near 36° F. during the summer.

Infection experiments were conducted in 1936 by inverting sporulating cultures over vetch plants infested by pea aphids in cages. In these experiments, *Empusa aphidis*, in culture since 1934, was compared with cultures newly made from field-collected aphids. The spores were projected in such large numbers, that the leaves of the small vetch plants as well as the aphids were well peppered with them in all cages. The highest percentage of aphids that were killed by the fungus in the 1934 strain was about 36, whereas an average of 95 per cent were killed by a fresh 1936 strain and by the natural fungus from an infected aphid which was introduced into one of the cages. Similarly a few experiments in 1935 had indicated a reduction in virulence in a strain that had been grown artificially for only 1 year. Hence there appears to be no doubt that culture on artificial media caused the fungus to lose its virulence for aphids. This was not true with the entomogenous fungi of hyphomycetous forms, such as *Metarrhizium*, *Beauveria*, and *Spicaria rileyi* (Farlow) Charles.

It should be noted that the entomophthorous fungi on aphids died soon after spore production and that this spore production was usually over in one or two nights. After that, saprophytic fungi, such as *Mucor*, developed on the carcases. It seems likely that this would be generally true of the fungi in this group, although in species that produce resting spores these spores probably remain viable over a long period. Hence the practice of collecting insects killed by these fungi would probably be useless as a means for disseminating the fungous disease except by resting spores. If this were to be attempted it would be necessary to air-dry the dying and recently dead insects before the hyphae emerged. As for the potentialities of the resting spores in those species in which they occur, no one has yet determined when or under what conditions they would germinate. It seems likely that there would be a considerable time lag before germination, perhaps several months.

We may conclude that the chances for artificially inducing epizootics of fungous disease among insects by utilizing the Entomophthoraceae are much less than in the case of the hyphomycetous fungi, because the Entomophthoraceae lose their virulence for insects after a short period of culture on artificial media.

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The first annual meeting of the new Southwestern Branch of the Association will be held on March 1 and 2 in Dallas, Texas. Hotel Headquarters will be in the Hotel Adolphus where Mr. P. J. Reno, of the Hercules Powder Company, will be in charge of local arrangements.

CHANGE IN MEETING PLACE OF THE NORTH-CENTRAL STATES BRANCH

A change has been made in the meeting place of the North Central States Branch which was scheduled earlier to meet at East Lansing, Michigan. The meeting will be held in Toledo, Ohio, on March 21 and 22, 1951. Dr. Roy L. Janes, of Michigan State College, will be the local Chairman.

Translocation of Parathion from Foliage Applications

FLOYD F. SMITH, *Bureau of Entomology and Plant Quarantine, U.S.D.A.*, and PAUL A. CLIFFORD, *Food and Drug Administration, Federal Security Agency*[1]

Questel & Connin (1947) reported that parathion in the soil is taken up by roots of corn plants and is translocated to the aerial parts in sufficient quantity to be toxic to larvae of the European corn borer *Pyrausta nubilalis* (Hbn.). Granger and Leiby (1949) also reported that parathion was translocated from the soil to the foliage of bean and nasturtium in sufficient quantities to be toxic to the Mexican bean beetle, *Epilachna varivestis* Muls., and to the black bean aphid, *Aphis rumicis* (L.), respectively.

The present studies, made during the summer of 1948, were planned to determine the extent to which parathion is translocated from the treated foliage of certain vegetables to the edible portions—i.e., roots, younger leaves, or fruits—of the same plants. In greenhouse tests the experiments were designed to preclude the absorption of parathion through the roots. In field tests the run-off from treated foliage and its possible absorption by roots in the contaminated soil were not prevented. Limited greenhouse tests were also made to determine the rate that parathion disappeared from rose leaves following applications of aerosol.

MATERIAL AND METHODS. *Greenhouse Tests.*—In the greenhouse tests the vegetables were planted on May 5, as seeds or as small seedlings, in 1-gallon crocks containing vermiculite, which is micaceous rock expanded by heating. The crocks were of a type used for plant-nutrient culture studies, having drainage holes on one side near the bottom.

The plants were fed at weekly intervals with a commercial nutrient mixture,[2] diluted at the rate of 1 ounce to 15 gallons of water. The bottom hole was stoppered, and the crock filled to near the top with the nutrient solution. After a few minutes the stopper was removed and, as the solution drained out, the rooting medium was aerated. Water was added once or twice each week, the drainage hole being left open. All plants grew vigorously except radish, which tended to go to seed, and cucumber, which may have been adversely affected by the warm temperatures.

For each crock a celluloid cover was made with a corked opening in the center for introducing nutrient solution or water, Each plant was trained to grow close to the edge of the crock, and the cover was cut to fit around each stem. Cotton was wrapped around the stem to allow for expansion. and the opening in the cover sealed with adhesive cellulose tape. This seal prevented material from running down the stem and reaching the rooting medium. The plants were supported by stakes held in place by wires around the crock.

To prevent contamination the young central leaves of the leafy vegetables were enclosed in parchment bags or celluloid cylinders before the parathion was applied. The fruits of tomato, bean, and similar plants were bagged at blossom time to protect the edible portions from contact with treated leaves.

The parathion was applied at weekly intervals beginning June 11 and continuing until July 23. The leaves of treated plants were either dipped in or painted with a frequently agitated water suspension that contained 2 pounds of 25 per cent wettable powder per 100 gallons. The treatments were carried out by two people wearing rubber gloves; one held the crock and staked plant in proper position, and the other dipped the leaves in the parathion suspension or painted it on the leaves. After treatment the plants were placed in an inclined position so that excess material dripping from the leaves did not fall on the cover or crock.

In addition to the designed translocation tests from treated foliage, analyses were made of rose leaves from greenhouses treated with parathion aerosol.

Field-Plot Tests.—In the field-plot tests on vegetables a water suspension containing 2 pounds of 25 per cent parathion wettable powder per 100 gallons was applied to both sides of the leaves, until the foliage dripped, by means of a knapsack sprayer operated at 80 pounds' pressure.

Strawberries were treated with 1 pound

[1] The writers are indebted to Neil W. Stuart, plant physiologist, Bureau of Plant Industry, Soils and Agricultural Engineering, for advice on growing the plants in an artificial medium and for the loan of certain equipment.

[2] *Hyponex*.

of either a 15 per cent or a 25 per cent wettable powder per 100 gallons applied by a power sprayer at the approximate rate of 125 gallons of spray per acre.

Upon reaching maturity the fruits, leaves, or roots were harvested 6 days after the last application of insecticide in a manner to prevent contamination from treated foliage, placed in cellophane bags, and held in a refrigerator until analyzed for parathion.

Parathion Determinations.—The colorimetric procedure for determining parathion devised by Averell & Norris (1948) was used in this study. The samples of plant material were handled in either of two ways: (1) The material was all allowed to air-dry and was then ground to 20-mesh size in a laboratory mill. Weighed portions, usually 5 grams, were then extracted with benzene in a Soxhlet extractor for 2 to 3 hours. Extracts were made to volume and clarified, and aliquot portions were carried through the procedure. Strawberry caps, cherry leaves, pea vines, and rose leaves were handled in this manner. (2) All other samples were handled in the fresh state. They were first pureed with about an equal weight of water in a Waring blendor; then benzene at the rate of 100 ml. per 100 grams of original sample was added and the mixture pureed for an additional 5 minutes. A creamy emulsion usually resulted. The emulsion was centrifuged, and a fairly large fraction appeared as an aqueous layer at the bottom of the centrifuge bottle. This layer was siphoned off, and from 50 to 100 grams of anhydrous sodium sulfate was mixed with the remainder either by shaking the stoppered bottles or by stirring with a stout glass rod. Further centrifuging produced a clear benzene layer, which could be decanted or siphoned off.

Sometimes as little as one-half the total benzene added was recovered as an extract. However, results are based upon the supposition that emulsions were homogeneous and that each milliliter of benezene extract represented 1 gram of sample. Evaporation losses were disregarded. Again the benzene extracts, sometimes highly colored, were clarified, and portions (usually 50 ml.) were carried through the procedure.

In both cases extracts were decolorized and dewaxed by shaking for 5 minutes in a glass-stoppered flask with a mixture of anhydrous sodium sulfate 10 parts, Attapulgus clay (200 mesh and finer) 5 parts, a diatomaceous earth 5 parts, and activated charcoal 2 parts. This absorbent mixture was added in the proportion of 10 to 15 grams per 100 ml. of extract. After filtration from the mixture, the benzene extracts usually were nearly colorless, but not all the waxy material was removed.

Final photometric colors were usually read in a neutral wedge photometer, a 5.0-cm. cell and filter centering at 560 millimicrons being used. Use of a longer cell increased the sensitivity of the instrument, and comparison was made with a color chart containing a standard curve for reading 1 to 100 micrograms of parathion. Colors were stable and reproducible. A number of extracts gave colors too faint for accurate photometric measurement. These extracts were transferred to 50-ml. tall-form Nessler tubes, and the parathion content was estimated by visual comparison with standards.

The test is so delicate that standard colors made with 0.5, 1.0, 1.5, 2.0, etc., micrograms of parathion show a nice regression when read in this manner. To eliminate turbidity in the final colors, it was necessary to use carefully redistilled benzene and freshly prepared reagents (especially the ethylenediamine coupling reagent). It was found advantageous, likewise, to mix about 200 mg. of diatomaceous earth into the cooled solutions after the reduction of parathion with zinc and prior to the filtration into the 50-ml. volumetric flasks. None of the reduced parathion was lost in this process.

With certain vegetables, particularly cabbage and to a lesser extent collards and kale, an interfering pink color developed during the zinc reduction. This interfering color is likely to appear when crucifers are analyzed (Averell & Norris 1948). After treatment with the diatomaceous earth and upon standing (sometimes overnight), this pink color usually disappeared and water-white filtrates were obtained. However, in one sample of cabbage the color persisted and vitiated the parathion test. In all cases reported here no value for parathion is given unless (1) filtrates were clear and colorless and (2) the characteristic pink color developed immediately upon addition of the coupling reagent.

Table 1.—Results of analyses of greenhouse-grown vegetables given various applications of parathion sprays.

Vegetable	Applications	Parts of Plant Analyzed	Parathion in Sample (Green Weight)	
			Untreated Plants	Treated Plants
	Number		*P.p.m.*	*P.p.m.*
Beans, lima	6	Beans, from bagged pods	0.0	0.0
	6	Beans, from unbagged pods	—	11.2
Beans, snap	4	Pods, bagged	.0	.04
Beet	3	Roots	.0	.01
Cabbage	6	Heads, bagged	.04	.13
Cabbage, Chinese	4	Heads, in celluloid cylinder	.0	.15
Chard	3	Central leaves, in celluloid cylinder	.0	.34
Collards	4	Upper leaves, in celluloid cylinder	.15	.31
Cucumber	4	Fruits, bagged	.4	Trace
Kale	4	Central leaves, bagged	.06	.18
Pepper	7	Fruits, bagged	Trace	Trace
Radish	4	Roots	.0	Trace
Tomato	6	Fruits, bagged	.0	.0
	7	Fruits, bagged	.0	.0

A limited amount of work was done in unsuccessful efforts to eliminate the interfering color. Thus, treatment with a little activated charcoal after the zinc reduction and prior to filtration of the mixture, oxidation with a few drops of saturated potassium permangenate solution at this point, or extraction of the filtrate with a small portion of amyl alcohol, removed the interfering color but resulted in more or less complete loss of the reduced parathion.

In recovery experiments to check on the efficiency of the analytical method where 1 to 2 p.p.m. of parathion was added to samples of unsprayed strawberries and cabbage, 80 to 88 per cent was recovered.

RESULTS.—Table 1 gives the amounts of parathion in 12 treated vegetables grown in the greenhouse in covered crocks to prevent absorption by the roots. The parts analyzed were protected from direct application of the chemical as previously described.

No parathion was found in the shelled lima beans or fruits of tomato. Traces—less than 0.01 p.p.m.—were recorded for cucumber, pepper, and radish. Snap beans and beets showed 0.04 and 0.01 p.p.m. Cabbage, Chinese cabbage, Swiss chard, collards, and kale showed 0.13 to 0.34 p.p.m., but untreated cabbage, collards, and kale also showed an interfering pink color and indicated readings up to 0.15 p.p.m.

Of particular interest is the high reading for parathion in shelled lima beans from unbagged pods that received four applications of the parathion suspension. Beans from bagged pods on the same plant showed none. The technic used in the greenhouse tests obviates as far as possible the accidental contamination of the samples with parathion. The plants were grown in a greenhouse with side ventilators open at all times and with top ventilators open except during storms, so that the concentration of vaporizing parathion was always at a minimum. However, absorption of any dilute parathion vapors was not precluded.

In the field tests snap beans sprayed once, while in blossom, showed no parathion in the harvested crop. Those beans from plants given two weekly applications, the last when the oldest beans were 1.5 inches long, showed 0.02 p.p.m. of parathion in samples at harvest 8 days later. Bagged cucumbers from plants given two weekly applications of parathion, the first as the blossoms dropped after pollination and the second a week later, showed less than 0.02 p.p.m. of parathion. Bagged summer squashes from vines given two applications showed a parathion content near 0.01 p.p.m.

TESTS ON PEAS.—Alaska peas in a field at Greensboro, Md., were sprayed on May 21, 1948, with 2 pounds of 25 per cent parathion wettable powder per 100 gallons at the rate of 200 gallons per acre. At this rate 1 pound of parathion was applied per acre. At the time of treatment

the peas were in blossom or with small pods. As the treatment was made a few days later than is considered best for commercial control of pea aphids, the usual interval between treatment and harvest was reduced.

Samples of vines from 1-square-yard areas were cut at the ground level and weighed on June 4, or 14 days after the spray had been applied. The average green weight of the vine samples was 3 pounds 9 ounces, or 1,616 grams, per square yard. At the rate of 1 pound of parathion per acre, each square yard received 0.094 gram. If all had been retained by the treated vines until harvest, 58.2 p.p.m. of parathion would have been present. Actually the entire vines and pods at harvest held about 0.05 p.p.m. on a green-weight basis. No trace of parathion was obtained in an analysis of peas shelled by cutting off the ends of the pods and removing the contents so as to avoid contamination.

On the basis of this test with an excessive dosage of parathion, it does not appear that any serious residue problem will arise on harvested peas even though surface contamination is possible during the vining process.

TESTS ON STRAWBERRIES.—Field plantings of strawberries at the Plant Industry Station, Beltsville, Md., were sprayed on October 10, 1947, with 1 pound of 15 per cent parathion wettable powder per 100 gallons of water for the control of aphids and spider mites. A second application of 1 pound of 25 per cent parathion wettable powder was made on April 20, 1948, at which time the plants were in full bloom and a few berries were present. A third spray of the same strength as the second was applied to another plot on May 18 as the first berries began to ripen. All sprays were applied at the rate of 125 gallons per acre.

Table 2 shows the results of analyses for parathion on 2-quart samples of fruits harvested on May 18 (plot 1) and May 26 (plot 2) that received the two early applications of parathion and also on fruits that received the third application near maturity (Plot 3).

The results indicate a small but definite quantity of parathion in the outer as well as the inner portions of the berries and a greater amount in the stems 4 to 5 weeks after the last application of parathion.

Table 2.—Parathion present in samples of strawberry fruits from treated plots.

PART OF FRUIT ANALYZED	PLOT 1	PLOT 2	PLOT 3
	P.p.m.	*P.p.m.*	*P.p.m.*
Whole fruit, unstemmed	0.08	—	—
Whole fruit, stemmed	—	Trace	—
Cut fruit, outer portion	.02	—	0.23
Cut fruit, inner portion	.02	—	.10
Stems and caps	.5	0.18	3.05

Larger quantities were found in the corresponding portions and in the stems of fruit on which an application was made 8 days before harvest.

Analyses of fruit from untreated strawberry plots showed no parathion.

TESTS ON ROSE LEAVES.—Better Times rose bushes grown in a greenhouse were treated with parathion in aerosols at the rate of 1 gram per 1,000 cubic feet for control of the two-spotted spider mites (*Tetranychus simaculatus* Harvey) and the leaf roller (*Platynota flavedana* Wkm.). Samples of the leaves were collected at intervals, and each lot, weighing 18 grams, was kept overnight in a bell jar of 0.17 cubic feet capacity, together with a bean plant infested with spider mites. The decline in toxicity to mites is closely correlated with loss of parathion, as evidenced by the chemical analysis (Table 3). In 9 days the material had practically dissipated.

SUMMARY.—In greenhouse tests parathion was applied to the foliage of 12 vegetables at a concentration of 2 pounds of 25 per cent wettable powder per 100 gallons. The edible portions of the plants were protected from contamination by enclosure in parchment bags or celluloid

Table 3.—Parathion residue on greenhouse rose leaves treated with aerosols, and its fumigating action on spider mites.

DATE OF TREATMENT	INTERVAL BETWEEN TREATMENT AND SAMPLING	RESIDUE IN GREEN LEAVES	MORTALITY OF SPIDER MITES
	Days	*P.p.m.*	*Per cent*
June 8	3	17.1	88
	6	4.08	11
	9	1.05	0
17	4	33.1	77
	7	12	16

cylinders. Analyses of the protected portions showed no parathion in shelled lima beans or tomato; less than 0.01 p.p.m. in cucumber, pepper, and radish; 0.04 and 0.01 p.p.m. in snap beans and beets; and 0.13 to 0.34 p.p.m. in cabbage, Chinese cabbage, Swiss chard, collards, and kale. Untreated checks of cabbage, collards, and kale also showed a reading of 0.15 p.p.m. for parathion. Unbagged shelled lima beans showed 11.2 p.p.m. of parathion, whereas bagged beans from the same plants showed none.

In field tests snap beans sprayed while in blossom showed no parathion in harvested beans, and those sprayed when beans were 1.5 inches long showed 0.02 p.p.m. at harvest 8 days later. Bagged cucumbers and summer squashes showed 0.02 p.p.m. parathion after two applications. At harvest time, 14 days after treatment, shelled peas showed no parathion, whereas the vines showed 0.05 p.p.m.

Strawberries showed 0.02 p.p.m. of parathion in fruits when treated in the blossoming stage or in the young fruit stage. Application 8 days before harvest resulted in 0.23 p.p.m. in the berries and 3.05 p.p.m. in the stems and caps.

In a greenhouse test where parathion had been applied as an aerosol, rose leaves had a maximum of 33 p.p.m. 4 days later. In 9 days the material had practically dissipated. Fumigating action of the residues on rose leaves against the two-spotted spider mite (*Tetranychus simaculatus* Harvey) decreased with the dissipation of the parathion.

The results of greenhouse and field tests indicated that in some vegetables parathion maybe translocated from treated foliage, but only in minute quantities. Absorption of vapors was not precluded.

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The Fungicidal Effect of Sulfur on Entomogenous Fungi Attacking Purple Scale

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For a number of years, it has been an accepted fact among research workers, growers, and other observers that residual materials, such as sprays containing copper, zinc, or lime, or the accumulation of other residues as road dust, fertilizer, *et cetera*, will cause an abnormal increase in the purple scale, *Lepidosaphes beckii* (Newm.), insect populations on Citrus trees in Florida. Thompson (1935) has pointed out that on plots sprayed with either 10 or 20 pounds of hydrated lime per 100 gallons of water, purple scales and Florida red scales, *Chrysomphalus aonidum* (L.), showed a marked increase in comparison with non-sprayed controls. Fawcett (1912) reported, as early as 1912, that the use of commercial lime-sulfur somewhat diminished the number of scale insects, but later caused a slight increase. Miller (1937) observed in 1937 that a very small quantity of sulfur on citrus trees would occasionally result in severe scale populations. Osburn & Spencer (1938) demonstrated that, following sprays containing lime-sulfur and wettable sulfur plus various adhesives, marked increases were observed in scale populations. Spencer (1939) also reported that heavy residues from sprays containing calcium hydroxide produced significant scale increases on citrus trees and that the presence or absence of copper in these sprays was not important. In comparing the effects of different concentrations of cupric carbonate and ammonia, copper sulfate and lime, and aluminum sulfate plus lime, Holloway & Young (1943)

concluded that the amount of residue might be more important than the type of residue. Griffiths & Fisher (1949) reported that any of the materials they used (pyrophyllite, wettable sulfur, basic copper sulfate, lime, and zinc sulfate) resulted in increases in purple scale populations which were greater than those which occurred on non-sprayed controls. They concluded that where amounts of residue are similar the chemical nature of the residue is the determining factor in the extent and duration of the abnormally high populations.

Although it has been demonstrated (Fisher *et al.*, 1949), under Florida conditions, that the endoparasitic chytrid, *Myiophagus* sp. Thaxter, is a major factor in the natural control of purple scale insects, it has been apparent that the natural control of these insects is probably determined by a number of factors. The writers have reported (1949, 1950) one other fungus, *Hirsutella Besseyi* Fisher, which attacks and apparently kills first instar purple scale nymphs. Whether or not this fungus is actually responsible for the periodic epizootics of the young scales remains to be proved; however, it is associated with the disease which causes high mortalities of the young insects, and in making scale counts it is used as an indicator of this particular disease."

The fungicidal affect of sulfur on chytridiosis was first observed by the writers in 1947. It has been shown (1949) that in plots which were sprayed twice in the spring with wettable sulfur, the percentage of mortality due to chytridiosis was somewhat retarded until the middle of the summer; however, these plots had population decreases at the same time as the non-sprayed controls. Since sulfur is used on citrus for the control of rust mites, *Phyllocoptruta oleivora* (Ashm.), the experiments reported here were designed for the purpose of determining the fungicidal effects of various sulfurs on the parasitic fungi, and the resultant effects on the purple scale populations.

METHODS, 1948.—The first experiment was begun in the middle of the summer of 1948 at which time the purple scale populations were decreasing. Three plots, wettable sulfur, lime-sulfur, and a non-sprayed control were used. Each of the three plots consisted of three Pineapple orange trees. The treated plots were

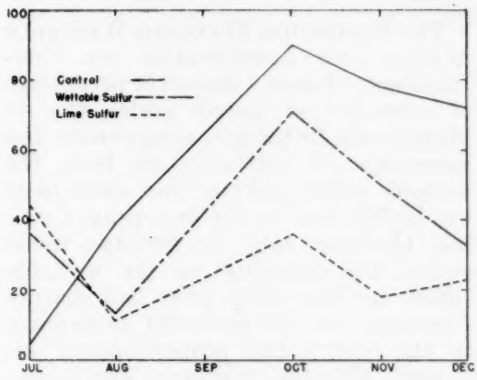

FIG. 1.—Percentage of mortality of *Lepidosaphes beckii* (Newm.) due to *Myiophagus* sp. Thaxter, 1948.

sprayed with 20 gallons of spray per tree on July 19 and each week thereafter for a total of 4 weeks in order to accumulate residual materials. The lime-sulfur plot was first sprayed with 2 gallons of liquid lime-sulfur per 100 gallons of water; but in the second and subsequent sprays the amount of lime-sulfur was reduced to 1 gallon per 100 gallons of water (a total equivalent of 12.5 pounds of sulfur). The wettable sulfur plot was sprayed each time with 5 pounds of 92 per cent wettable sulfur per 100 gallons of water (total of 18.4 pounds of sulfur). The amount of lime-sulfur was reduced to prevent foliage and fruit injury. Purple scales were counted prior to the first spray applications, and additional counts were made at regular intervals. Counts were also made to determine the percentage of mortality[1] due to chytridiosis throughout the same period. Scale counts were based on the microscopic examination of the scales on a quarter of the surface of 50 leaves selected at random from each tree. Each leaf was recorded as infested or non-infested. Identifiable male scales were not included in scale counts. Chytridiosis incidence was based on the examination of 20 randomly selected scale-infested leaves from each tree. Counts were made of the number of scales parasitized by the chytrid as well as the number of living scales present. Only females in late second or third instars were included in this count since it is only in these stages that chytridiosis can be easily recognized.

[1] $\frac{\text{Scales affected with chytridiosis}}{\text{Scales affected with chytridiosis} + \text{living scales}} \times 100.$

THE FUNGICIDAL EFFECT OF WETTABLE SULFUR AND LIME-SULFUR ON CHYTRIDIOSIS.—Figure 1 shows the percentage of mortality of purple scales due to chytridiosis. In the pre-spray counts, the percentage of mortality on both the wettable sulfur and the lime-sulfur plots was higher than on the non-sprayed control. However, after the first two treatments, the mortality on the wettable sulfur and lime-sulfur plots had sharply decreased, but the mortality percentage on the control had proportionately increased. After the treatments were terminated, August 16, the mortality increased on all three of the plots. Although the mortality percentage increased on all plots through October, the fungicidal effect of the sulfur was evident throughout the duration of the experiment. The mortality percentage was higher on the non-sprayed control than on the sprayed plots. Following the spraying treatments, the mortality in the wettable sulfur plot was consistently higher than in the lime-sulfur plot. The differential fungicidal effect between the wettable sulfur and the lime-sulfur was probably due to a higher fungicidal activity of the lime-sulfur.

Figure 2 shows the per cent decrease of quarter-leaves infested with purple scale. Although all of the scale populations were decreasing, the differential decrease is readily apparent. The non-sprayed control decreased more rapidly than did the wettable sulfur plot which, in turn, decreased more rapidly than the lime-sulfur plot. During November and December, the differences between the control and the wettable sulfur plots were not significant. Throughout the experiment, the decrease in the lime-sulfur plot was comparatively small.

A comparison of Figures 1 and 2 shows, in each plot, that as the percentage of mortality rises the scale population decreases. As the scale population decreases the percentage of mortality decreases. In December, there was a small increase in the scale populations which was followed in January by a similar increase in the percentage of mortality (not shown in the figures).

Table 1.—Total amount of materials used and pounds of sulfur in each treatment per 100 gallons of water for five experimental treatments.

TREATMENT	TOTAL AMOUNT PER 100 GAL.	POUNDS OF SULFUR PER 100 GAL.
Lime	10	0
Wettable Sulfur	10	9.2
Lime-Sulfur	2.9 gal.	7.25
Sulfur Paste	13.6	9.1
Non-sprayed	0	0

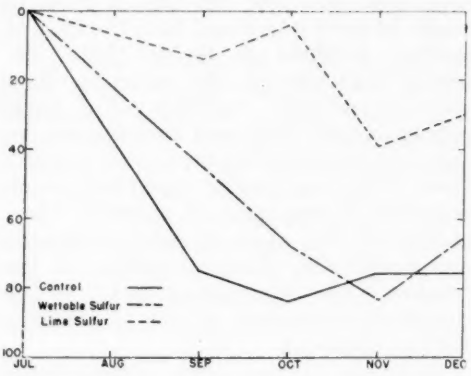

FIG. 2.—Percentage decrease of quarter-leaves infested with *Lepidosaphes beckii* (Newm.), 1948.

METHODS—1949.—The second phase of this work was begun in the spring of 1949 in a block having purple scale populations which were comparatively low and stable. This experiment was designed not only as a repetition and as an enlargement of the preceding experiment, but also to compare fungicidal residues with an apparently inert residue. Four treatments, hydrated lime, wettable sulfur, lime sulfur, sulfur paste,[1] and two non-sprayed controls were used. After setting an arbitrary figure of 10 pounds per 100 gallons of water for the lime and wettable sulfur treatments, equivalent amounts of residues were used in the lime-sulfur and sulfur paste treatments (see Table 1). The number of pounds of sulfur per 100 gallons of water is given in table 1. The plots, consisting of three trees each, were chosen at random in a block of Pineapple oranges. Sprays were applied at the rate of 25 gallons per tree, on April 27, May 26, June 29, and July 28. Purple scales were counted prior to the first spray applications and additional counts were made at monthly intervals. Counts were also made to determine the number of scale-infested leaves which had scales parasitized by *Myiophagus* sp. and nymphs

[1] *Magnetic 70*, sulfate paste, a commercial mixture consisting of: 69% sulfur, 3% wetting agent, and 28% water. Supplied by the courtesy of Stauffer Chemical Company, Apopka, Florida.

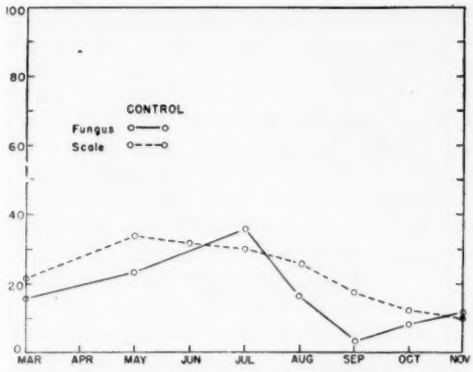

FIG. 3.—Average of two non-sprayed control plots. Per cent of purple scale-infested quarter-leaves, and per cent of infested leaves with one or more scales per leaf infected by the entomogenous fungi, *Myiophagus* sp. and *Hirsutella Besseyi*.

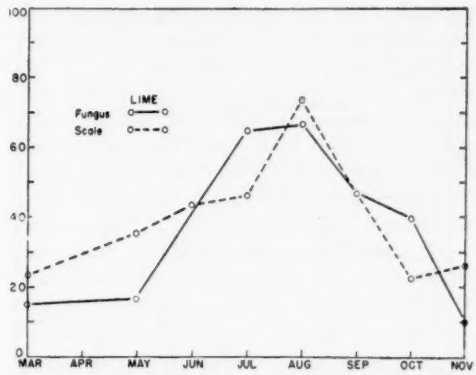

FIG. 4.—Lime-sprayed plot. Per cent of purple scale-infested quarter-leaves, and per cent of infested leaves with one or more scales per leaf infected by the entomogenous fungi, *Myiophagus* sp. and *Hirsutella Besseyi*.

attacked by *Hirsutella Besseyi* throughout the same period. Scale counts were made in the manner previously described. Entomogenous fungi counts were based on the examination of 20 randomly selected scale-infested leaves from each tree. Each leaf was recorded, as to the presence of either or both *Myiophagus* sp. and *Hirsutella Besseyi*, as infected or non-infected. This method of counting and recording made it possible to count all stages of scales rather than only second and third instars. In the figures which follow, the total per cent of infested leaves with scales infected by both fungi are used.

THE EFFECT OF LIME AND VARIOUS SULFURS ON ENTOMOGENOUS FUNGI AND CHANGES IN THE SCALE POPULATIONS.—Figure 3 shows an average of two non-sprayed control plots. The purple scale populations in the controls reached their maximum summer infestation at the end of May; this maximum was followed by an increase in the per cent of entomogenous fungi. As the scale mortality increased, the scale population decreased. In August and September, there was a sharp decrease in scale mortality. Counts made in October and November show the scale population to be approximately two-thirds of the original population count in March. In general, the purple scale population in these plots exhibited a small increase in the early part of the summer before the beginning of the rainy season. After the rainy season began, the entomogenous fungi showed further increases, and the scale population gradually decreased throughout the summer and fall. The scale population trends of the control plots vary from typical non-sprayed orange trees (1949) in that the scale population reached its peak in late May rather than in late June or July. However, the fungus trends are typical (1949), and either sampling error or the fact that these populations remained comparatively low throughout the summer may account for the atypical early summer infestation.

The purple scale infestation in the lime-sprayed plot (Fig. 4) reached its maximum infestation in August, three weeks after spraying was terminated, and in October had decreased to the same point as the original infestation. In the lime-treated plot, as in the control, the per cent of entomogenous fungi followed the insect population. Although the presence of residue apparently caused the exceedingly high population on the trees sprayed with lime, the scale population and fungus trends were typical of trees sprayed with a non-fungicidal material. Usually, on non-sprayed trees or trees sprayed with a non-fungicidal material, periods of scale population decrease or low incidence are generally preceded by or coincident with a high percentage of fungi; scale populations which have decreased are followed by decreases in the percentage of fungi. Since the behavior of the scale population and the fungi were typical, it might be concluded that lime exerted little or no fungicidal effect on *Myiophagus* sp. and *Hirsutella Besseyi*. However, it should not be overlooked that any residue may exert

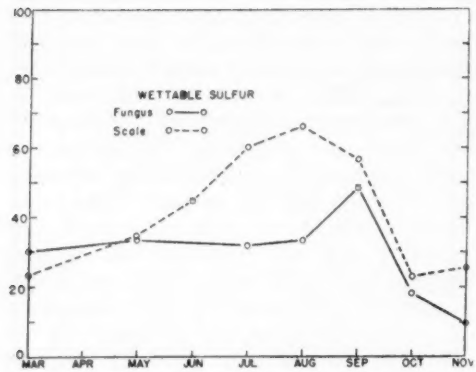

FIG. 5.—Wettable sulfur-sprayed plot. Per cent of purple scale-infested quarter-leaves, and per cent of infested leaves with one or more scales per leaf infected by the entomogenous fungi, *Myiophagus* sp. and *Hirsutella Besseyi*.

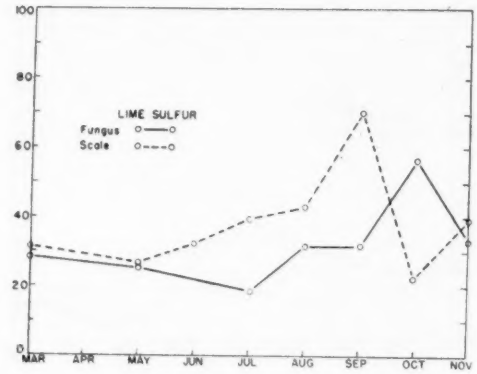

FIG. 6.—Lime-sulfur-sprayed plot. Per cent of purple scale-infested quarter-leaves, and per cent of infested leaves with one or more scales per leaf infected by the entomogenous fungi, *Myiophagus* sp. and *Hirsutella Besseyi*.

a mechanical effect on the zoospores of *Myiophagus* by impeding their progress in swimming (1949), and thereby providing protection for the scale insects.

Figure 5 shows the per cent of purple scale and of fungi in the wettable sulfur-sprayed plot. As in the lime-treated plot, the scale population reached a peak during August, three months later than the control. This gradual and high increase in the scale population was not directly followed by a gradual increase in the entomogenous fungi, as was true in the control and lime plots, but, rather, the fungi remained in a fairly stable percentage until September. The per cent of fungi sharply increased in September. Following this increase, the scale population and the per cent of fungi dropped sharply in October. At this time, the scale population had decreased to its original level. In a comparison of the wettable sulfur, lime, and control plots, it would appear that the wettable sulfur had some fungicidal effect on the entomogenous fungi. Although the fungicidal effect was not enough to completely inhibit the fungi, it was sufficient to prevent the fungi from showing a significant increase until a month and a half after the final spray.

The scale population in the lime-sulfur-treated plot (Fig. 6) did not reach a peak until September. This peak was reached three months later than in the control, and one month later than in the lime and wettable sulfur plots. It should be noted that until August the increases in the scale population were small in comparison with the lime and wettable sulfur plots. Thompson (1935) has reported that purple scale infestations were held in check or reduced by three lime-sulfur sprays 6 weeks apart. This would account for the low increases in the scale population throughout the spraying period. The per cent of fungi decreased during the spraying period, but increased after the final treatment. In October, a sharp rise in the per cent of fungi was coincident with a marked decrease in the scale population.

During August the scale population in the sulfur paste-sprayed plot (Fig. 7) reached the highest peak of all treatments included in this study. This plot was char-

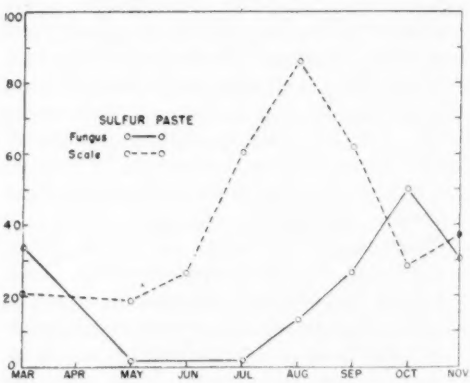

FIG. 7.—Sulfur paste sprayed plot. Per cent of purple scale-infested quarter-leaves, and per cent of infested leaves with one or more scales per leaf infected by the entomogenous fungi, *Myiophagus* sp. and *Hirsutella Besseyi*.

acterized by rapid increases of the scale population until a peak was reached, and similar rapid decreases following that point. The per cent of entomogenous fungi from the time spraying was begun until two weeks after spraying was terminated, was almost negligible, but from August through October there was a continual increase in the incidence of fungi. This increase in fungi was coincident with the decrease of the scale population.

DISCUSSION.—Figures 1 and 2 show that the purple scale population on the wettable sulfur-sprayed plot decreased more rapidly than did the population in the lime-sulfur-sprayed plot, and that the mortality caused by chytridiosis was higher where wettable sulfur was used than where lime-sulfur was used. It should be noted that the lime-sulfur plot received a total equivalent amount of 12.5 pounds of sulfur, whereas the wettable sulfur plot received 18.4 pounds, or almost a third more sulfur. If the total amount of residue was the only factor involved in scale population changes, as has been indicated by some investigators (Holloway & Young 1943; Spencer 1939), then, the scale population in the wettable sulfur plot should have maintained a higher level than the population in the lime-sulfur plot. However, it is seen that this was not true, but, rather, the lime-sulfur resulted in a higher population. During the period of spraying (the month of July), the chytridiosis mortality decreased in both the wettable sulfur and the lime-sulfur plots. After the final spray, both treatments showed chytridiosis increases; however, these increases were greater in the wettable sulfur plot. Throughout the duration of the experiment, the fungicidal effect of both sulfurs, particularly lime-sulfur, was evident.

In a comparison of figures 5 and 7, it is seen that two materials, wettable sulfur and sulfur paste, with similar amounts of total sulfur (Table 1) exhibited widely different effects on the entomogenous fungi. In the wettable sulfur treatment, the fungi increased slightly, but were maintained at a fairly stable level until six weeks after the final spray. The fungicidal effect of the sulfur paste reduced the number of fungi to an almost negligible amount throughout the spraying period. At its peak, the scale population in this plot was more than 20 per cent higher than in the wettable sulfur plot. At that time and throughout the fall the fruit and the leaves of the sulfur paste-sprayed trees were densely covered with scales, whereas, the infestation on the wettable sulfur-sprayed trees was very light. Unfortunately, the scale-counting method used does not show differences between light, general infestations and general infestations of very heavy densities.

In the lime-sulfur plot (Fig. 6) apparently the scales were partially controlled (Thompson 1935) by the spray applications. Six weeks after the final treatment the population sharply increased to a degree which exceeded that in the wettable sulfur plot, even though less sulfur (Table 1) was used. The entomogenous fungi showed decreases during the period of spraying, but, following that period, they increased slightly and continued increasing.

The lime-sprayed plot and the control plot (Figs. 3 and 4) had scale populations and entomogenous fungi which were fairly typical (Fisher & Griffiths 1949), respectively, of inert-material-sprayed trees and non-sprayed trees. Apparently the lime had no fungicidal effect on either *Myiophagus* sp. or *Hirsutella Besseyi*.

A comparison of all of the sulfur plots shows that the amount of sulfur (Table 1) applied on the lime-sulfur plot was less than on the wettable sulfur plot, and the amounts applied to the wettable sulfur and sulfur paste plots were almost equal. However, the density of the scale populations on the three plots, in descending order was: sulfur paste, lime-sulfur, and wettable sulfur. The explanations for these differences are not fully known, but the particle sizes may account for the differential fungicidal effects of sulfur paste and wettable sulfur. Hamilton, *et al.* (1943) found that the fungicidal effectiveness of wettable sulfurs was inversely proportional to the size of the particles. They also concluded that the finer particle materials were more toxic because of the larger surface area exposed to the water film in which the spores germinate. According to their measurements, the average particle size of sulfur paste is 8.3–9.7μ. The average particle size of the dry wettable sulfur which was used in these experiments is 28μ. The size of the free sulfur particles precipitated by the polysulfide sulfur in the liquid lime-sulfur

which was used is not known, but in these experiments lime-sulfur had more fungicidal effect than the wettable sulfur and less than the sulfur paste. McCallan (1949), in a recent summary of the fungicidal action of sulfurs, has reported that the decomposition of lime-sulfur polysulfides results in the precipitation of finely divided free sulfur. Also he states that the fungicidal action of the sulfurs which are included in this study are in the end dependent on free elemental sulfur. Since a lower amount of lime-sulfur exhibited more fungicidal effect than did the amount of wettable sulfur which was used, it is probable that the actual particle size of the free elemental sulfur precipitated from lime-sulfur is smaller than the particle size of the wettable sulfur.

In all of the sulfur plots it should be noted that significant increases in the fungi did not occur until after the spraying treatments were terminated. The amount of *Hirsutella Besseyi* was low in all cases, for it occurs only on purple scale nymphs. However, the total numbers of *Myiophagus* and *Hirsutella* were used since they followed similar patterns, and also because the nymphs and adult scales were used to compute the per cent scale-infested quarter-leaves.

SUMMARY AND CONCLUSION.—On Citrus in Florida, it was found that wettable sulfur, lime-sulfur, and a sulfur-paste exerted fungicidal effects on the entomogenous fungi, *Myiophagus* sp. Thaxter and *Hirsutella Besseyi* Fisher. Under Florida conditions, *Myiophagus* is a major factor in the natural control of purple scale insects, *Lepidosaphes beckii* (Newm.), and *H. Besseyi* is regularly found to be associated with purple scale nymph mortality.

Wettable sulfur exhibited a comparatively small fungicidal action, whereas, sulfur paste was fungicidal to a high degree, and lime-sulfur was intermediate. Hydrated lime apparently had little or no fungicidal effect.

In general, purple scale infestations were most severe on the trees sprayed with sulfur paste. The treatments in descending order of scale infestations were: sulfur paste, lime-sulfur, wettable sulfur, lime, and the non-sprayed control. The non-sprayed control trees had much less scale than did the other treatments.

From the standpoint of obtaining as much natural control of purple scale insects as possible by the use of entomogenous fungi, it appears that wettable sulfur would be more desirable for the control of rust mites, *Phyllocoptruta oleivora* (Ashm.), than would lime-sulfur or sulfur paste.

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SCIENTIFIC NOTES

Races of Apomictic Parasitic Hymenoptera Introduced into California[1]

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The biological control of scale insects on citrus in California has involved the introduction, from various parts of the world, of parasitic Hymenoptera that are completely apomictic. In such Hymenoptera the eggs are unfertilized, and establishment of the species is not handicapped by the need for mating.

The phenomenon of apomixis is common among the species of parasitic Hymenoptera. Although the eggs of completely apomictic species are not fertilized, these species may be bisexual as well as unisexual. Geographical races based on such sexuality are known. For example: *Hemiteles areator* and *Eupelmella vesicularis* are bisexual in Europe and unisexual in North America (Musebeck & Dohanian 1927); *Leucospis gigas* is bisexual in North Africa and unisexual in Europe (Berland 1934); *Trichogramma embryophagum* is bisexual in North America and unisexuall in Europe, and *Thripoctenus brui* is bisexual in Japan and unisexual in Europe (Clausen 1940). Collections of *Prospaltella perniciosi* Tower made along the eastern coast of North America and sent to California in 1943 and 1946 revealed that this species is bisexual in the North Atlantic states and unisexual in Georgia (Tower 1914, Rice 1937).

In some apomictic species bisexuality is seasonal, as in *Harmolita grandis, Ditropinotus aureoviridis,* and *Microctonus brevicollis* (Phillips & Emery 1919, Phillips & Poos 1921, Phillips 1920). This may be a nutritional effect. The bisexuality of the gall-forming eurytomid *Trichilogaster acaciae-longifoliae* varies with different species of host plants (Noble 1940). Flanders (1945) has found that the production of unisexual or bisexual broods by *Habrolepis rouxi,* an apomictic parasite of the California red scale, *Aodiniella aurantii,* depends on the nutrition of the parent females during development. More than a hundred consecutive generations of *H. rouxi* have been produced in the insectary without mating. The mating instinct of the female is entirely lacking; that of the male is present but is weak and limited to following the female without touching her.

In 1948 and 1949 two host-determined races of apomictic Hymenoptera, *Aspidiotiphagus citrinus* and *Prospaltella perniciosi,* parasitic on red scale, were introduced into California from China. A host-determined race of *A. citrinus* has been present in California for many years as a parasite of several diaspine scale insects, but not of red scale; and a race of *P. perniciosi* (Fig. 1) parasitic on San Jose scale, but not on red scale, was introduced into California from Georgia in 1943 (Flanders 1944b) and is now widely established.

The red-scale-feeding race of *Aspidiotiphagus citrinus* introduced from China in 1948 has been propagated in large numbers on red scale in the insectary. This form appears to be indistinguishable morphologically from *A. citrinus* (Craw), the yellow-scale-inhabiting form. The two forms can be distinguished only on the basis of a difference in their host relationships. The yellow-scale-inhibiting form does not reproduce on red scale in California, either under natural conditions out-of-doors or under artificial conditions in the insectary. In the practical work of biological control the two forms are treated as separate entities, since they are the fundamental units with which we work.

The history of the red-scale-inhabiting and yellow-scale-inhabiting forms of *Aspidiotiphagus* is confused. The latter form first came into prominence in the San Gabriel Valley in 1890, andw as described and named by Craw as *citrinus* in 1891. The history of the red-scale-inhabiting form dates back to 1900, when George Compere shipped, from China, to Alexander Craw in California, a small orange tree heavily infested with red scale. Craw (1901–02) reported rearing a total of 647 parasites from the red scale on this tree. These parasites were released in orange orchards in Los Angeles and Orange counties. Craw described the species as "a parasite like *Aspidiotiphagus* only smaller." The most valuable clue to the identity of this parasite is provided in an unpublished letter from Dr. L. O. Howard to Alexander Craw, dated Washington D.C., May 22, 1902: "I shall be interested in your reply to my letter in which I reiterated my opinion that your red scale parasite is nothing but what I have all along taken to be *Aspidiotiphagus citrinus* Craw."

Since Howard wrote his letter, entomologists have repeatedly identified the red-scale-inhabiting species of *Aspidiotiphagus* as *citrinus,* and have regarded it as the same as the yellow-scale-inhabiting species. F. Silvestri, in 1924–25, and Harold Compere, in 1932, reared the red-scale-inhabiting race from material collected in China, but made no attempts to introduce it into California, where it was thought to be already established.

The red-scale-inhabiting race of *Aspidiotiphagus citrinus* was reported from South Africa in 1902 by C. P. Lounsbury, who stated that a parasite appearing to be *A. citrinus* was first noticed at Bathurst five years before, and then again in 1902; but while upwards of half the scales on some twigs were affected, no appreciable advantage derived from its presence.

In Australia a form of *Aspidiotiphagus* parasitic in red scale was repeatedly reared by N. S. Noble. In 1934, in a letter to Harold Compere, Noble wrote in regard to the red-scale-inhabiting form: "As far as I can determine *Aspidiotiphagus australiensis* Gir. appears to be identical with *Aspidiotiphagus citrinus* Craw."

Whether or not the red-scale-inhabiting *Aspidiotiphagus* under consideration is the same as that described by Girault under the name *Aspidiotiphagus australiensis* is a matter of conjecture. The description of *A. australiensis* is based on a specimen collected at Nelson (Cairns), Queensland, and does not provide characters for its separation. The host of *A. australiensis* is not known.

The lack of a formal scientific name to designate the red-scale-inhabiting form of *Aspidiotiphagus* may cause some inconvenience in making an index to the literature. It is thought to be more scientific, however, to state that the red-scale-inhabiting from represents an entity of unknown systematic posi-

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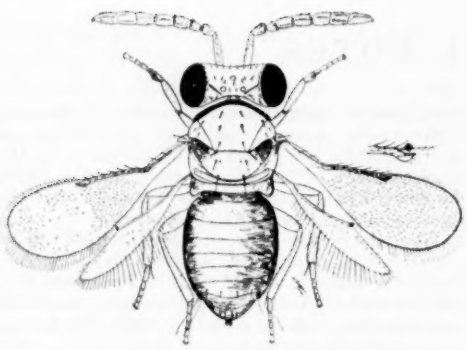

FIG. 1.—Female of *Prospaltella perniciosi* Tower. (Drawing by Harold Compere.)

tion, and to leave it unnamed, than to attempt to determine its relationships by guess, and to apply a formal scientific name having a definite meaning which may prove to be incorrect and misleading.

The race of *Prospaltella perniciosi* parasitic on red scale was introduced into California from Formosa, propagated in the laboratory, and released in large numbers. It now appears to be permanently established. Although *P. perniciosi* was obtained first from San Jose scale collected in Massacnusetts (Tower 1914), circumstantial evidence indicates that it is of Asiatic origin, as is its host the San Jose scale. The red-scale-inhabiting form of *Prospaltella* and its host are also believed to be of Asiatic origin. Presumably, the two forms of *Prospaltella* evolved in China. The form parasitic in San Jose scale was no doubt carried by commerce to North America, where it became established on the eastern seaboard of the United States, and to Brazil, South America.

Nakayama (1921) reported *Prospaltella aurantii* from *Aspidiotus perniciosus* in the vicinity of Tokyo. It is probable that this parasite was *P. perniciosi* and not *P. aurantii*, which occurs in California, where it does not attack San Jose scale. Kuwana (1901) observed that in some localities in Japan nearly all the female scales contained the immature stages of an aphelinid parasite. This, also, may have been *Prospaltella perniciosi*, since no other species is known to develop in stages of San Jose scale distinguishable as females.

The first demonstration of the fact that a species of parasitic Hymenoptera may consist of host-limited races occurred when an amphimictic species, *Comperiella bifasciata* How., was introduced into California from China and Japan for the control of red scale (Flanders 1944a; Smith 1942). The parasites from both China and Japan oviposited readily in red scale. The form from China reproduced in large numbers generation after generation, but the form from Japan did not. The development of the Japanese form, unlike that of the Chinese form, was restricted to a very limited stage of the red scale, so that the average progeny per parent was only one. The average progeny per parent fro the Chinese form was 25.

The discovery that *Comperiella bifasciata*, *Prospaltella perniciosi*, and *Aspidiotiphagus citrinus* consist of host-limited races finally establishes the principle that in parasite importation programs cognizance must be taken of this phenomenon. The host specificity of a parasitic species in one region is not necessarily the same as that in another.

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A Comparison of DDT-Resistant and Non-Resistant House Flies

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With respect to toxicological studies on house flies, very little has been reported on the comparative morphology and physiology of DDT-resistant and nonresistant strains. Wiesmann (1947) reported morphological and physiological differences between a DDT-resistant strain of house flies (Arnaes strain) from Sweden, and a nonresistant laboratory strain (Basle strain) from Switzerland. In comparison with the Basle strain, the Arnaes strain showed, for example, greater cuticular pigmentation, stiffer tarsal bristles, shorter and wider tarsal segments, thicker tarsal cuticular and intersegmental membranes, and a lower susceptibility to immobilization by heat and cold and to narcosis by methyl acetate.

This study was undertaken to determine whether or not similar differences might be concerned between a DDT-resistant house-fly strain (Bellflower strain) from southern California and a nonresistant laboratory strain, toxicological studies on which were reported by March & Metcalf (1949). An exact duplication of Wiesmann's investigations was not attempted, but the Bellflower and laboratory strains were compared in the following manner.

Studies of the gross morphology of the two strains did not reveal any differences. A microscopic comparison of tarsal lengths and widths was made from tarsi prepared by the standard KOH method and mounted on slides. Ten individual tarsal segments were measured for the determination of each average dimension shown in table 1. In general the tarsi of the Bellflower strain tended to be slightly shorter than those of the laboratory strain, the first tarsal segment of the midleg of the females being the only exception. The tarsal widths, however, were more variable, those of the Bellflower strain being slightly narrower in 14 cases, equal in 3 and wider in 13. The differences in the dimensions of the tarsal segments of the two strains were statistically valid in most cases, however they do not approach the significantly large and uniform differences found by Wiesmann for the Arnaes and Basle strains.

Longisections of the first tarsal segment of the right foreleg from females of both strains were made by the general paraffin method. Safranin was used to stain the tissues, leaving the endocuticula translucent and the exocuticula a contrasting black or brown. Measurements of 10 tarsal segments showed the average range in thickness of the cuticula to be 8.5 to 12.0 microns for the Bellflower strain, and 8.2 to 11.7 microns for the laboratory strain. The least significant mean difference ($t_{.05}$) was 1.2 microns. Since Hayes & Liu (1947) had found no essential differences in house flies in the cuticular thickness of the different tarsal segments or of the corresponding tarsal segments of the two sexes, measurements were completed for only the single tarsal segment. Cross sections of the thorax showed that the cuticular thickness was of the same magnitude as for the tarsi.

The general vigor of the two strains was compared by the determination of the length of life cycle, average weight of individual flies, and susceptibility to immobilization by heat and cold. Both strains were reared under identical conditions at a temperature of 80° F. and a relative humidity of 40%, according to the standardized procedure described in Soap Blue Book (1947).

Analyses of records for 23 generations showed the average length of life cycle to be 18.1 days for the Bellflower strain, and 17.9 days for the laboratory strain. The least significant mean difference ($t_{.05}$ was 2.5 days.

Average weight of individual female flies determined over a period of 7 months from 10 groups of 20 flies each of uniform 3 to 4 day age was 18.2 mg. for the Bellflower strain and 18.2 mg. for the laboratory strain. The least significant mean difference ($t_{.05}$) was 2.9 mg.

The time in minutes for immobilization of 50 per cent of a test group of 10 flies of uniform 3 to 4 day age was determined at five temperatures for each sex.

Table 1.—Comparison of Bellflower and laboratory strains of house flies based on average length and width of tarsal segments.

Strain, Sex and Leg	Average Lengths (L) and Widths (W) in Microns for Following Tarsal Segments									
	1st		2nd		3rd		4th		5th	
	L.	W.	L.	W.	L.	W.	L.	W.	L.	W.
Bellflower ♀										
foreleg	639.2	97.0	281.7	93.3	248.8	84.5	213.0	75.6	255.9	68.0
midleg	926.6	99.3	381.8	95.0	277.4	86.8	208.7	80.9	270.2	73.3
hindleg	856.5	103.0	441.8	93.7	323.1	84.5	233.0	83.5	283.1	85.4
Laboratory ♀										
foreleg	677.6	102.6	340.3	95.4	271.7	8.18	230.2	74.3	271.7	68.0
midleg	920.9	100.3	408.9	95.0	301.7	84.5	225.9	75.9	274.5	65.3
hindleg	912.3	108.9	491.9	98.7	358.9	84.2	260.2	73.9	314.6	66.7
Bellflower ♂										
foreleg	659.2	88.8	324.6	89.4	274.5	83.2	228.8	76.9	255.9	83.6
midleg	895.1	92.4	387.5	89.8	293.1	82.5	225.9	76.9	278.8	79.2
hindleg	892.3	98.7	507.6	86.8	377.5	77.2	273.1	72.9	298.8	67.7
Laboratory ♂										
foreleg	727.8	99.7	364.6	95.0	301.7	84.5	248.8	79.5	277.4	77.6
midleg	912.3	92.4	417.5	90.1	320.3	81.8	243.1	75.9	283.1	73.6
hindleg	930.9	112.5	533.3	98.0	400.4	83.2	296.0	74.3	326.0	65.7

Table 2.—Comparison of Bellflower and laboratory strains of house flies based on average time in minutes for 50% immobilization by heat and cold.

Strain and Sex	Average Time in Minutes for 50 Per Cent Immobilization at Following Temperatures				
	60° C.	50° C.	45° C.	0° C.	−8° C.
Bellflower ♀	3.3	4.9	9.6	5.6	3.2
Laboratory ♀	3.3	4.9	7.9	5.6	2.9
Bellflower ♂	3.0	4.7	8.5	5.7	2.9
Laboratory ♂	2.8	5.5	7.9	5.4	2.9

Each test was replicated four times. The results are shown in table 2. The least significant mean difference ($t_{.05}$) between strains was 1.0 minute, thus except for the females at 45° C., there was no difference in the susceptibility of the two strains to immobilization by heat and cold.

This general comparative study indicates that thickness of the cuticula and general vigor are not concerned in the greater than 300-fold increase in resistance to DDT shown by the Bellflower strain. The differences in the dimensions of the tarsal segments of the two strains are not uniform enough or large enough to indicate that they contribute to the resistance.

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The Brown Dog Tick on Rats in Puerto Rico

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The relationship of the brown dog tick, *Rhipicephalus sanguineus* (Latr.), to domestic rats, the primary reservoir of infection of murine typhus fever, is of more than academic interest because that common tick is a demonstrated vector of at least two rickettsial diseases in the tropics. It has already been shown that this ectoparasite does indeed engorge on rats in nature at least in the nymphal stage. Thurman and Mulrennan, (1947) and experimentally Luttermouse (1947) induced the larvae to feed on shaven white rats. In view of these circumstances the tick is suspect as a possible vector of murine typhus, but speculation on its role in the epidemiology of the disease should be based upon data which indicate the degree that it infests rats. The purpose of this note therefore is to contribute to this end by presenting the results as regards *R. sanguineus* of a survey of 1326 rats made in the city of San Juan, Puerto Rico where both the tick and murine typhus commonly occur.

The 1326 rats were collected during the period January 17, 1946 through March 8, 1949, 938 rats originating from the residential section of the municipality of San Juan known as Santurce, and 388 rats from the business district known as San Juan proper. Of the 938 rats from Santurce 809 were *Rattus norvegicus* and 129 were *R. rattus*, while of the 388 rats from San Juan proper 354 were *R. rattus* and 34 were *R. norvegicus*. The rats from Santurce came from the poorer residences and their environs, but those from San Juan proper came from restaurants and similar establishments. These ecological differences between the two districts make it advisable to allude to the data as being from two separate surveys.

Material combed from 30 of the 938 Santurce rats, that is two individuals of *Rattus rattus* and 28 *R. norvegicus*, yielded 35 brown dog ticks (8 larvae, 9 nymphs and 18 adults) which gives an infestation per cent of 3.2 and an average per rat of .037. The maximum number of ticks taken from a single rat was three, but seldom was a rat infested by more than one tick. Only five of the 35 specimens collected showed certain evidence of engorgement; they were four adults and one nymph. Records of the occurrence of ticks on rats were obtained for each month of the year, but during different years; however, a statistically significant and seasonal variation in number or infestation per cent could not be ascribed to the ticks.

Two of the 388 rats from San Juan proper were found infested, both were *Rattus rattus* and had one tick each, an unengorged larva and an unengorged adult. The infestation per cent, therefore, was only 0.5 and the average number of specimens per rat only 0.005.

It is evident from the foregoing that the brown dog tick affects rats to only a minor degree; and it is unlikely that this ectoparasite could play a significant part in the transmission of murine typhus in Puerto Rico. Rats have not heretofore been recorded as hosts of the brown dog tick in Puerto Rico. Tate (1941) lists dogs, domesticated rabbits and occasionally man.

Thanks are due Dr. R. A. Cooley who in April 1946, kindly confirmed a determination of an unfed nymph as *Rhipicephalus sanguineus*.

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Contamination of Milk from DDT Sprays Applied to Dairy Barns[1]

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Studies made during 1948 indicated that DDT or TDE applied as residual sprays in dairy barns might contaminate milk with the insecticide. Precise evaluations of such contamination could not be made at that time, because the cattle milked in those barns were also being sprayed with the same insecticides for horn fly control. Therefore, early in 1949, in advance of the horn fly season, further tests were made on the effects of spraying dairy barns when the cows were not treated with an insecticide.

Three experiments were designed to determine whether the DDT was getting into the milk through careless handling of the milk and milking equipment, through inhalation, or through ingestion of the insecticide from contamination of the feed.

First it seemed desirable to know whether the DDT was actually secreted in the milk or was getting into it from careless handling.

A barn was sprayed with a wettable-powder suspension containing 2.5 per cent of DDT. A composite sample of milk taken on the morning of the spraying just before treatment contained 0.1 p.p.m. of DDT. Two days after the barn was sprayed samples of milk were taken from the 11 cows in the barn by milking directly into sampling bottles held to one side. Five samples were taken from the cows, each sample representing milk from 2 cows, and a sixth sample was taken from the eleventh cow. The dairy operator then milked the cows by machine, and a composite sample was taken from a 10-gallon container to represent the machine milking. Analyses of the hand-milked samples showed 1.0, 0.8, 0.6, 0.4, 0.2, and 0.6 p.p.m. of DDT. The machine-milked composite sample contained 0.6 p.p.m. of DDT. These results indicated that the cows were secreting the DDT in the milk.

A special experiment was designed to determine whether the cows were getting the DDT by inhalation. A barn that had never been sprayed with an insecticide was selected. The floor was covered with heavy kraft paper, and the walls and ceiling were sprayed with sufficient 2-per cent aqueous suspension of DDT to give a deposit of 200 mg. per square foot. About 15 minutes after the spraying the paper was removed and an 8- by 12-foot pen was erected in the center of the barn. A Jersey cow producing about 3 gallons of milk per day was milked and a control sample taken. The cow was placed in the pen and confined there all night, and a milk sample was taken the next morning. This procedure of confining the cow in the pen all night and collecting the milk sample in the morning was repeated until six samples were obtained. All samples, including the control, were analyzed for DDT, and all were negative.

A final experiment was set up to determine whether the DDT was getting into the milk from contamination of the feed as a result of spray in the feed troughs. Six dairy barns were selected. Three were sprayed without special care to keep drippings or fog from settling into the feed troughs. Reasonable care was used to avoid wetting of the feed troughs, and no spray was directed deliberately at the troughs or stanchions. The other three barns were sprayed with utmost care to avoid contamination. The feed troughs were masked by suspending sheets of heavy wrapping paper over the length of the manger and securing them laterally with masking tape to the top of the stanchions and the wall. Portable equipment was removed from the barns. The dairymen milkers were requested to use utmost care to avoid contamination.

All barns were sprayed with a wettable-powder suspension containing 2.5 per cent of DDT. The surfaces were sprayed only enough to obtain wetting with practically no run-off. Milk samples were taken at each barn before spraying and 1, 2, 6, and 14 days thereafter. Butterfat determinations were not made, but the average butterfat content of the samples collected the previous season from the same barns was approximately 4 per cent. The DDT determinations were made by the method of Schechter *et al.* (1947), and results are given in table 1.

Table 1.—Parts per million of DDT in milk samples taken after barns were sprayed with a wettable-powder suspension containing 2.5 per cent of DDT.

Days Spraying to Sampling	Troughs Exposed			Troughs Covered		
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
0 (control)	0	0	0	0	0	0
1	Trace	.24	.6	.1	Trace	0
2	0	1.3	1.4	.5	Trace	0
6	Trace	.2	.3	.3	0	0
14	Trace	.2	.1	.1	Trace	0
34	—	.1	—	—	—	—

Barn 1, which was sprayed with only ordinary care with feed troughs uncovered, had only a trace of DDT in the milk. After the experiment it was learned that the operator at this dairy washed the feed troughs with a hose immediately after the spraying was completed. Barn 4 showed some contamination of milk even though the feed trough was covered. The construction of the stanchions was such that it was impractical to cover the front of the feed trough. Although the top was shielded, it was possible for some fog to drift into it.

It appears that from ordinary spraying enough of the spray mist settles in the feed troughs for cows to ingest significant amounts of DDT, which is subsequently secreted in the milk. Contamination of the milk was avoided in two barns by thoroughly covering the feed troughs and at another by washing the feed troughs after the spraying was completed.

SUMMARY.—A study was made to determine the source of milk contamination when dairy barns were sprayed with 2.5 per cent of DDT. Three experiments showed (1) that the insecticide was actually secreted in the milk and did not get into it after milking due to mishandling of the milk or milking equipment, (2) that no contamination resulted from inhalation of the insecticide by the cows, and (3) the spray residues left on the feed trough resulted in the secretion of the insecticide in the milk.

[1] Presented before the Texas Entomological Society at Houston, Texas, January 1950.

Contamination of milk did not occur when troughs were completely covered during the spraying or when the troughs were washed after the spraying.

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An Anobiid Beetle Infesting Avocado Trees

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In the course of investigation of insect pests of avocado in southern California, it was noted that dead flower stalks and dried twig terminals were infested with an anobiid beetle, *Ozognathus cornutus* (Le Conte). This species has been known to be active on oaks, where it usually infests the twigs and supposedly exists also in the cynipid galls on these twigs (Fall, 1905). Eggs of the beetle have been found under the "Button" of lemon fruits and this species has been hatched and reared from the eggs.

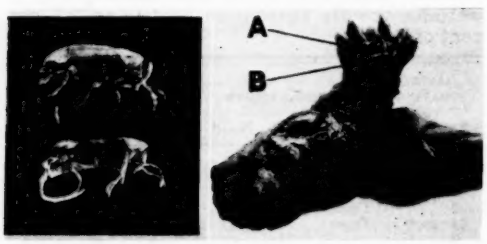

FIG. 1.—*Ozognathus cornutus* (Le Conte). Left: male, above, with mandibular horns, and female, below, with ovipositor extended. ×8.5. Right: exit hole in dead avocado terminal (A) and emerged adult immediately prior to flight (B). ×2.

While considerable numbers of these beetles are to be found in avocado plantings, they are considered to be of little or no importance as a pest.

There is some evidence that living tissue is attacked. After the inner contents of a dried terminal has been consumed, the larva will work into the living tissue, where it feeds enough to enable it to complete its life cycle.

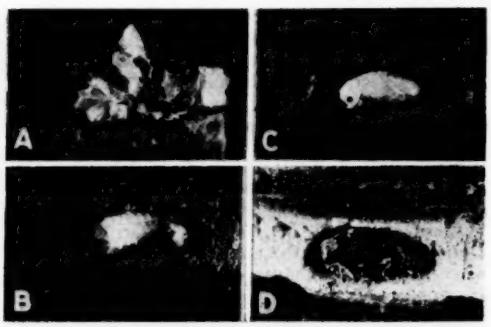

FIG. 2.—*Ozognathus cornutus* (Le Conte) in dead flower stalks of avocado. A, Eggs; B, Larva; C, Pupa; D, Adult. A, greatly magnified; B, C and D, ×8.5.

The adult beetles measure 2 to 2.8 mm. in length. They are rather stout, cylindrical, blackish brown, and would be very shiny were it not for the short pubescence. The males are characterized by a pair of short mandibular horns which are lacking in the female. The ovipositor of the female, when extended, is very long, measuring over half the length of the body (Fig. 1).

The eggs (Fig. 2 A) are greyish white, cylindrical, and are laid in a flat mass. The long ovipositor of the female makes it possible for the eggs to be deposited deep within cracks or under the dead terminal growth. The young, on hatching, start feeding in the surrounding dead tissue, where they soon fill the cavity with copious amounts of small fecal pellets. The developed larvae are usually found feeding singly in the flower stems or dead terminal growth. Pupation occurs within the cavity formed by these larvae. The adults then gnaw a round exit hole through which they escape and take flight (fig. 1 B).

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Codling Moth Oöcyte Studies

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During the summer of 1941 approximately 19,000 codling moths, *Carpocapsa pomonella* (L.), were trapped in bait pails in an 18-acre 34-year-old orchard containing 477 bearing apple trees near Martinsburg, W. Va. The varieties were York Imperial, Stayman Winesap, Ben Davis, and Black Twig. One bait pail was suspended in each bearing tree and was examined regularly for codling moths. Several different bait solutions were used throughout the season. There was no evidence that trapping the moths reduced the amount of codling moth injury to fruits. At harvest time about 70 per cent of the fruit showed worm and sting injury. Other work had been conducted in which bait pails also failed to show value in codling moth control. Thus, it was evident that large numbers of codling moths could be trapped with attractive baits and taken from an orchard without effecting a measurable reduction of wormy fruits.

A study of oöcyte production and maturation was undertaken as a possible explanation of why a catch of large numbers of moths in bait pails failed to have a greater effect on codling moth control. The information obtained might be useful in interpreting bait-trap catches for timing spray applications, since oöcyte production, egg deposition, and hatching follow in natural order. Therefore, oöcyte counts of moths trapped in bait pails were compared with similar counts of reared moths of various ages, to determine possible peaks in oöcyte production as related to seasonal temperatures and age of moths. Codling moths for this study were collected in 1942 during May, June, July, and August and were processed and preserved in alcohol until fall, when

the oöcyte counts were made. Work on oöcyte counts was not continued the following season because the work program was interrupted by the war.

METHOD.—Overwintering codling moth larvae that had been concentrated in rolls of 1-inch corrugated paper bands were reared to get spring-brood moths. Larvae taken in bands on infested trees were the source of moths of later broods. The rolls containing larvae were suspended in cylindrical screened cages 18 by 24 inches in natural orchard shale. The cages were fitted at the bottom with cloth sleeves tied to prevent the escape of moths and to provide a means of entrance. Moths were allowed to emerge in a cage from 6 P.M. until 7:30 A.M. The rolls were taken out to prevent further emergence. The moths were kept for 1, 3, 6, 9, and 12 days, and appropriate samples were taken for oöcyte counts. The foregoing procedure permitted a possible variance of 13.5 hours in the age of the moths used. Moths that had emerged over night were kept an additional 24 hours in the cage and then placed in fixing solution. Thus, some moths used for day-old counts may have been 37.5 hours old rather than 24. The same age variance applies to samples of the moths 3, 6, 9 and 12 days old.

Wild moths, or those occurring naturally, were collected daily from bait pails suspended in apple trees. All collections were made without regard to sex. To determine the sex ratio more accurately, more moths were collected than could be used in the oöcyte counts. When a collection was too large to permit the counting of oöcytes in each female, the total numbers of male and female moths were recorded for that collection.

A small shell vial containing a few cubic centimeters of Carnoy's fixing solution was used to collect moths from the cages. The open end of the vial was placed over a moth on the screen wall of the cage, and when the vial was moved the moth fell into the solution.

After the moths had been in Carnoy's fixing solution for 1 hour, their wings were removed. Then they were hydrated by immersion in successive changes of alcohol beginning with a 95 per cent concentration and decreasing the strength 10 per cent every 30 minutes through a 40 per cent solution. The moths were then stained by placing them in Grenacher's borax-carmine solution for 1 to 2 days. After being stained they were destained for 8 to 12 hours in 40 per cent acid alcohol, and then dehydrated in successive changes of alcohol by increasing the concentration 10 per cent every 30 minutes from 40 per cent through 70 per cent. The material remained in 70 per cent alcohol until oöcyte counts were made.

The moths were placed in a small petri dish partially filled with paraffin, and were dissected under a binocular microscope with 10× oculars and 55-mm. objectives, two needles being used in making the dissections. The abdomen of each female moth was opened ventro-longitudinally and the folds of the egg sac were carefully separated so that individual oöcytes could be counted.

Immature oöcytes took up the carmine stain and appeared as small to minute red bodies. Mature oöcytes did not stain, and appeared as white objects usually compressed into cylindrical shape. Chorion formation evidently prevented the stain from penetrating the mature oöcytes.

The egg sac becomes increasingly smaller toward its anterior attachment, and a point is reached where oöcyte differentiation can no longer be seen at the magnification in these experiments—about 40×. Counts of immature oöcytes given in this report should be considered as relative to the magnification used in counting, rather than as absolute.

Temperature readings for both day and night were taken from a hygrothermograph kept in a weather shed in an orchard. Average day temperatures were calculated from readings every 2 hours from 8 A.M. to 6 P.M. and average night temperatures from readings from 8 P.M. to 6 A.M. The temperatures are shown in the following tabulation:

	Average Mean Temperature	
Month	*Day*	*Night*
May	70	63
June	75	69
July	79	71
August	75	68

RESULTS.—The life span of caged moths decreased from an average of about 10 days in May and 9 days in June to 3 or 4 days in both July and August. Several 6-day old moths were collected in July, but they were weak and inactive and their tissues broke down in the staining and dehydration processes to such an extent that satisfactory oöcyte counts were not possible. One collection of 6-day old moths made in August was in suitable condition for oöcyte counts. Caged moths lived long enough to permit only two collections, both in May, of those as old as 12 days. In July all caged moths died within 4 to 5 days.

Table 1.—Ratio of female to male moths among those caught in bait traps and those reared in cages, by months.

MOTHS	MAY	JUNE	JULY	AUGUST	SEASONAL AVERAGE
Bait-trap catches	0.5	0.5	1.5	1.5	1.0
Reared in cages					
1-day-old	1.6	1.5	1.4	1.4	1.5
3-day-old	1.3	1.5	1.8	3.4	2
6-day-old	3	12.3	—	2	5.8
9-day-old	5.4	24	—	—	14.7
12-day-old	6	—	—	—	—

The numbers of oöcytes found per female moth are shown in Table 2 by months and by age group, where known. The number of moths examined varied from lot to lot, ranging from 18 6-day old moths reared in May to 132 wild moths captured in July.

Table 2.—Average number of oöcytes per moth, by age group.

AGE OF MOTHS (DAYS)	MAY	JUNE	JULY	AUGUST
Reared				
1	116	136	143	152
3	148	158	203	193
6	90	132	—	132
9	123	132	—	—
12	106	—	—	—
Wild	81	67	67	66

The data show that the total number oöcytes was markedly lower in wild female moths captured in bait pails than in reared moths, regardless of the age

of the latter or of the month. Among wild moths the total number of oöcytes per female was somewhat higher in May than in the warmer months. Among reared moths the 3-day old group had more oöcytes than any other age group, regardless of the month, but had fewer early in the season than later.

When only mature oöcytes are considered, the average for wild female moths for the season was only eight whereas in reared moths the largest numbers were found in 9-day old moths in May (51) and June 60) and in 3-day old moths in July (83) and August (85).

DISCUSSION.—The data given above indicate that oöcyte formation and differentiation proceed faster in warm than in cool weather, that oöcyte formation reaches a peak early in the life of the moths, that moths lay a considerable per cent of their eggs before being captured in bait traps, and that the number of the eggs laid by moths before being caught is moderately lower in the cooler early part of the season than later. The small number of mature oöcytes in wild moths indicate that they oviposited much more readily in nature than in confinement, and that the wild moths lay a considerable number of their eggs before their capture. While counts were not made of eggs deposited inside the cages, observations indicated that oviposition was light. No eggs were observed in cages containing moths 3 days old or less. It is not known what effect confinement and subsequent reduction in oviposition below normal have had on oöcyte formation. The light oviposition in cages does not completely account for the considerable drop in oöcytes in 6-day old confined moths below the number in those but 3 days old. The possibility of reabsorption of oöcytes is a suggested explanation.

The ratio of females to males was lower in moths taken in bait traps than in moths that emerged in the cages. Thus the bait traps apparently have permitted a greater proportion of females to remain in the orchard.

It is possible that not all the difference between the average number of oöcytes found in wild moths caught in bait traps and the average number found in reared moths may have been due to egg deposition by the wild moths before capture, but apparently enough of the difference was due to this fact to explain why large bait-trap captures failed to be reflected in marked reductions in worm-injured fruit. If moths deposit most of their eggs before entering bait traps, their capture can have little influence on the percentage of worm-injured fruit.

Fall Armyworm Control[1]

L. P. DITMAN, *University of Maryland, College Park*

During the summer of 1949 an early outbreak of fall army worm, *Laphygma frugiperda*, occurred in parts of Maryland. A block of sweet corn on the Maryland Experiment Station farm was found to be seriously infested on August 1. Eight different treatments, including water sprays of DDT and parathion, and DDT aerosols (see table) were applied on August 2. The water sprays were applied with equipment designed for corn borer control, the boom being equipped with 3 nozzles per row. Aerosols were applied with a covered two-row dis-

[1] Paper No. A278, Contribution No. 2224 of the Maryland Agricultural Experiment Station (Dept. of Entomology).

Table 1.—Treatments and their effectiveness on fall army worm larvae in young sweet corn.

TREATMENT	NO. PLOTS	LARVAE PER 100 PLANTS AUG. 2	LARVAE PER 100 PLANTS AUG. 4	REDUCTION ON TREATED PLOTS	PLANTS INJURED AUG. 2-8	CONTROL BASED ON CHECK
DDT 50% W.P., 2 lb.[2]; water 100 gal. at 200 gal. per acre. 400 lbs. pressure.	2	91.6	0	100%	10.5%	83.2%
DDT 30% emulsion, 1 qt.,[2] at 200 gal. per acre. 400 lb. pressure.	3	95.3	1.9	97.7	3.3	94.7
Parathion 25% W.P., 1 lb.[2]; at 200 gal. per acre. 400 lb. pressure.	2	99.3	0	100	5.5	91.2
DDT 10% aerosol, 15 lb. per acre.	2	112.4	28.2	65.5	22.5	64.0
DDT 5% aerosol, 15 lbs. per acre.	2	133.0	25.7	68.6	23.0	63.2
DDT 30% emulsion, 1 qt.; water 10 gal. Size T 2 nozzles.[1] 7.5 gals. per acre, 100 lbs. pressure.	3	76.8	23.6	71.2	14.0	77.6
DDT 30% emulsion 1 qt.; water 30 gals. Size T 6 nozzles, 15 gals. per acre, 100 lbs. pressure.	3	76.9	14.2	82.6	11.6	81.4
DDT 30% emulsion, 1 qt.,[2] Size T 24 nozzles, 80 gals. per acre, 100 lbs. pressure.	3	76.4	2.5	96.9	5.3	91.5
Check	2	134.6	81.9		62.5	

[1] Size refers to Spraying System's solid cone nozzles. Gallons per acre estimated on basis of nozzle output, pressure, and speed.
[2] % 100 gallons water.

penser. The corn plants were 12 to 15 inches high at time of treatment. In examining the field after a 48 hour period, it was observed that nearly grown larvae which were feeding deep in the heart of the curl and protected by a layer of grass were the most difficult to kill. Because of the rapid growth of plants at this time, old and new injury was readily distinguished within a week and observations gave a further check on the effectiveness of treatments. Data pertaining to the experiment and results are given in the table.

Though these plots of corn were later used for experimental treatment for European corn borer and corn earworm control, it was obvious that effective control of the fall army worm at this early period resulted in greatly increased yields of corn.

Summer Oil Sprays to Control Blueberry Bud Mite

W. E. TOMLINSON, JR., *New Jersey Agricultural Experiment Station*[1]

The blueberry bud mite, *Aceria vaccinii* (Keifer), has been troublesome on certain varieties of cultivated blueberries in New Jersey for a number of years. Where the bud mite infestation is severe the blossom buds are so deformed by their feeding that the blossoms do not open and, therefore, do not set fruit. In less severe infestations the blossoms may open and set fruit, but the berries are often ruined by the roughening and blistering of the skin caused by the feeding of the mites.

Spraying experiments were started against this mite in the spring of 1947 during the delayed dormant and pre-bloom period. Although a fair kill of bud mites was obtained by some materials at this time, it was noticed that bud mite injury was not alleviated, indicating that the buds had been damaged before the sprays were applied. Bud examinations during the summer, fall and winter of 1947 showed that mites were present under the leaf or fruit bud scales in all stages throughout the entire year, and that injury to the fruit buds starts in late summer, soon after the buds are formed, and continues throughout the fall and winter on warm days. However, during late summer and early fall the mites are usually still feeding under the outer bud scales only.

In 1948 infested Cabot bushes were sprayed directly after harvest was completed in August, before the mites had penetrated the buds to the flower parts. A summer oil (Orthol K[2]) applied at 2 per cent actual oil content looked promising on the basis of counts made a week after the spray was applied. The bud mite infestation declined naturally throughout the sprayed area during the fall and winter, and the following spring (1949) no differences could be noticed between the sprayed plots and the unsprayed check plots.

In early October of 1949, Harding bushes were sprayed with a summer oil[3] diluted with water to contain 3 per cent actual oil. Three rows of 50 plants each were sprayed twice, 10 days apart; three other rows of 50 plants each received the first application only; and three rows were left unsprayed. A week after the second application, bud examinations indicated that bud mite control was excellent. An examination of these plots during the blooming period in May, 1950, demonstrated the effectiveness of the oil sprays, as table 1 indicates:

Table 1.—Effect of autumn spray on mite injury to blossoms the following spring.

TREATMENT	NO. PLANTS	BLOSSOM CLUSTERS SHOWING GROSS FEEDING INJURY
2 applications 3% summer oil[2]	150	1
1 application 3% summer oil[2]	150	18
Check	150	505

CONCLUSION.—Practical control of the blueberry bud mite may be obtained with a post-harvest application of a summer oil at a 3 per cent oil dilution without injury to the fruit buds. Sprays containing 2 per cent summer oil also have looked promising, but more tests with this strength oil are needed to demonstrate whether it will give the desired results with a single application.

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University—the State University of New Jersey, Division of Horticulture.
[2] Orthol K, California Spray-Chemical Corporation.
[3] Orthol D-soluble, California Spray-Chemical Corporation.

Tests of Insecticides to Control Boll Weevil, Cotton Aphid, and Two-Spotted Mite

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Experiments with calcium arsenate, mixtures of calcium arsenate and organic insecticides, and various organic insecticides for control of the boll weevil, *Anthonomus grandis* Boh., the cotton aphid, *Aphis gossypii* Glov., and the two-spotted spider mite, *Tetranychus bimaculatus* Harvey, were conducted during 1949 at Tallulah, Louisiana. The results are briefly discussed herein.

In each experiment the plots were arranged in randomized blocks with four replications of each treatment. Each plot was 12 rows wide and of sufficient length to contain 0.1 acre or more. All infestation and yield records were made on the four center rows. Thus four buffer rows surrounded each area where records were obtained.

Boll weevil infestation records were made by examining 100 squares in each plot. Aphid infestation records were made by counting the aphids on one square inch of leaf surface near the center on the under side of the fourth leaf from the top of the plant. Aphids were counted on 100 leaves in each plot. Counts of the two-spotted spider mite were made in the same manner as aphid counts.

All insecticide applications were made with a single-nozzle rotary hand duster during the early morning, from daybreak to 8 a.m. The quantity of insecticide applied to each plot was determined by weighing the dust gun before and after each application.

Infestation and yield records obtained in the four experiments are given in table 1.

EXPERIMENT 1.—Chlordane mixtures, in the various concentrations and combinations, were not so effective as the other treatments in this experiment. The following treatments gave good control of the

[1] The authors were assisted in the field by G. L. Garrison, W. C. Johnson, E. P. Thomas, and A. J. Graham.

Table 1.—Results obtained in four experiments with various insecticides for control of the boll weevil, the cotton aphid, and the two-spotted spider mite. Tallulah, Louisiana, 1949.

Insecticide Formulation	Pounds per Acre	Seasonal Average		Mites per Sq. In. on One Date	Seed Cotton per Acre	
		Weevil-Punctured Squares	Aphids per Square Inch		Total	Gain Over Check
					Pounds	*Pounds*
Experiment 1, beginning at 13% boll weevil infestation						
Untreated check	—	48%	2.78	1.56	1272	
Calcium arsenate	8	31	7.89	1.43	1559	287
Calcium arsenate and nicotine 2%, alternated with calcium arsenate alone	9	33	1.84	.98	1761	489
Chlordane—						
15% plus sulfur 40%	11	33	.81	1.16	1485	213
10% plus sulfur 40%	11	39	1.97	.40	1534	262
5% plus sulfur 40%	11	41	2.47	1.49	1491	219
10% plus sulfur 40% plus DDT 5%	11	30	1.83	1.14	1453	181
Benzene hexachloride gamma 3% plus DDT 5% plus sulfur 40%—						
Alone	9	27	.13	1.30	1611	339
Alternated with calcium arsenate	7	33	2.25	.66	1696	424
Alternated with 2 applications of calcium arsenate	7	21	1.84	2.46	1901	629
Alternated with 3 applications of calcium arsenate	7	33	2.06	.78	1679	407
Toxaphene 20% plus sulfur 40%	12	28	.24	.54	1586	314
Minimum significant difference, 5% level	—	8	2.27	—	266	
Experiment 2, beginning at 22% boll weevil infestation						
Untreated check	—	56	1.57	1.62	1640	
Calcium arsenate	8	33	7.08	.78	2070	430
Calcium arsenate and nicotine 2%, alternated with calcium arsenate alone	9	50	.74	1.08	2286	646
Benzene hexachloride gamma 3% plus sulfur 40%—						
Alone	8	29	.07	1.17	1886	246
Plus DDT 5%	9	34	.17	1.70	2101	461
Calcium arsenate and a mixture of benzene hexachloride gamma 3%, DDT 5%, and sulfur 40%, as needed	8	38	.75	.80	2071	431
Toxaphene						
40% in clay	8	27	.06	.38	2122	482
20% in pyrophyllite	12	26	.06	.56	2081	441
Chlordane—						
20% in clay	5	34	.81	2.09	1773	133
10% in pyrophyllite	11	41	1.72	1.42	1783	143
Minimum significant difference, 5% level	—	9	.86	—	258	
Experiment 3, beginning at 22% boll weevil infestation						
Untreated check	—	58	1.93	.98	1537	
Calcium arsenate	8	42	7.48	.51	1743	206
Aldrin 2%	14	24	.52	3.85	2050	513
Dieldrin 1.5%	15	29	.38	4.35	1804	267
Chlordane—						
10%	10	33	.81	2.49	1824	287
10% plus sulfur 40%	11	38	1.05	1.17	1876	339
Toxaphene—						
20%	11	33	.09	1.53	1671	134
20% plus sulfur 40%	11	36	.11	.13	1835	298
Benzene hexachloride gamma 3% plus DDT 5%—						
Alone	9	33	.11	2.60	1937	400
Plus sulfur 40%	9	39	.15	1.25	1691	154
Minimum significant difference, 5% level	—	8	1.02	—	282	

Insecticide Formulation	Pounds per Acre	Seasonal Average: Weevil-Punctured Squares	Seasonal Average: Aphids per Square Inch	Mites per Sq. In on One Date	Seed Cotton per Acre: Total	Seed Cotton per Acre: Gain Over Check
					Pounds	*Pounds*
Experiment 4, beginning at 10% boll weevil infestation						
Untreated check	—	46%	7.12	1.63	1540	
Calcium arsenate—						
Special A	7	31	8.65	2.51	1547	7
Special A plus benzene hexachloride gamma 1%	7	24	3.66	1.47	1737	197
Regular plus benzene hexachloride gamma 1%	12	19	10.34	.45	1725	185
Special A plus parathion 1%	7	24	1.22	.53	1693	15x
Regular	9	25	11.56	.17	1726	188
Regular plus parathion 1%	8	29	7.44	.09	1825	285
Special B	10	28	9.92	.97	1679	139
Special B plus benzene hexachloride gamma 1%	10	18	2.50	.00	1939	399
Special B plus parathion 1%	12	23	.09	.05	1950	410
Benzene hexachloride—						
Special gamma 3% plus DDT 5% plus sulfur 40%	12	21	.07	.26	1746	206
Regular gamma 3% plus DDT 5% plus sulfur 40%	13	23	.14	.07	2025	485
Gamma 5% plus DDT 5% plus sulfur 30.7%[1]	9	16	.04	—	2035	534
Gamma 5% plus DDT 10% plus sulfur 21%[1]	9	24	.11	—	1819	318
Minimum significant difference, 5% level	—	8	4.52	—	—	

[1] Applied on only two blocks. Records were not used in analysis of variance.

two insects and the spider mite, and a substantial gain in yield: A mixture of calcium arsenate plus 2 per cent of nicotine alternated with calcium arsenate alone; a mixture containing sufficient benzene hexachloride to give 3 per cent of the gamma isomer, DDT 5 per cent, and sulfur 40 per cent, when used alone or when alternated with various applications of calcium arsenate; and toxaphene 20 per cent plus sulfur 40 per cent.

EXPERIMENT 2.—Benzene hexachloride gamma 3 per cent plus sulfur 40 per cent gave good control of the boll weevil, the cotton aphid, and the two-spotted spider mite, but gave a very small gain in yield over the untreated check; however, this mixture was much more effective in increasing the yield when 5 per cent of DDT was added. A mixture of calcium arsenate plus 2 per cent of nicotine alternated with calcium arsenate, calcium arsenate alone, and a mixture of benzene hexachloride gamma 3 per cent plus DDT 5 per cent plus sulfur 40 per cent, as needed, against cotton aphids gave good control of the two cotton insects and the mite, as well as very satisfactory gains in yield over the check. Toxaphene 40 per cent in clay applied at 3.2 pounds per acre per application, and toxaphene 20 per cent in pyrophyllite applied at 2.4 pounds were equally effective. Chlordane 20 per cent in clay applied at 1 pound per acre per application, and chlordane 10 per cent in pyrophyllite applied at 1.1 pounds were equally effective against boll weevils. However, chlordane was much less effective than most of the other insecticides included in this experiment.

EXPERIMENT 3.—A dust containing 2 per cent of aldrin gave good control of the boll weevil and the cotton aphid, but not of the spider mite. This dust also gave a greater gain in yield over the check than any other insecticide included in the experiment. A 1.5-per cent dieldrin dust was not quite so effective as the 2 per cent aldrin dust, and its use was followed by a build-up of mites. Chlordane, toxaphene, and a mixture of benzene hexachloride gamma 3 per cent plus DDT 5 per cent were each used alone and with 40 per cent of sulfur added. The mixtures without sulfur were as effective against mites and in increasing the yields as were mixtures containing sulfur.

EXPERIMENT 4.—The two brands, A and B, of special low-lime calcium arsenate and regular calcium arsenate were equally effective. The special calcium arsenates plus benzene hexachloride gamma 1 per cent were much more effective in preventing an aphid build-up than a similar mixture with regular calcium arsenate. The special calcium arsenates plus parathion 1 per cent gave good control of the cotton boll weevil, the cotton aphid, and the two-spotted spider mite, as well as substantial gains in yield over the check. A mixture containing a special benzene hexachloride gamma 3 per cent plus DDT 5 per cent plus sulfur 40 per cent was no more effective than a similar mixture but with regular benzene hexachloride. Mixtures containing benzene hexachloride gamma 5 per cent and either 5 per cent or 10 per cent of DDT plus sulfur were very effective against the two cotton insects, and each gave very substantial increases in yield.

Sprays for Fig Scale Control

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On the basis of work done from 1944 to 1947, Barnes & Stafford (1949) reported on the economic importance and life history of the fig scale, *Lepidosaphes ficus* (Sign.), in California. They reported control with dormant oil emulsion sprays or light grade summer oil applied in May. In 1948, certain growers stated that the suggested control measures were inadequate. Also, some concern was expressed that oil sprays might contribute to the occurrence of bacterial blight of Adriatic figs. In 1949, experiments were set up to test the proposed dormant oil control measures in three fig growing areas and to investigate further the use of parathion in fig scale control.

METHODS.—Replicated plots were set up in a Kadota fig orchard in Tulare County and in Adriatic fig orchards in Fresno and Merced Counties. The following data were taken on the 1948 growth in the Adriatic orchards: 1. length of twig growth and 2. number of live and dead adult female scales. In the Merced County orchard, the average number of live scales per inch of twig was 3.6 on February 21, and the per cent dead scales was 32.7. In the Fresno County orchard, the average number of live scales per inch of twig was 5.6 on March 11. The per cent dead scales was 22.7. The mortality was much greater than in previous years (5 to 7 per cent) and was probably due to very cold weather. Though no such data were taken in the Tulare County orchard, observations showed it to have about the same degree of infestation as the Merced County orchard.

In each location the same dormant oil was used either as tank-mix, dormant emulsive oil (about 98 per cent actual oil), or dormant oil emulsion (about 80 per cent actual oil). The oil was supplied and formulated by the same commercial concern.

Previous work had shown that the best time for parathion control of fig scale was before any of the first brood females had reached the adult stage but late enough in the spring so that the overwintered females were nearly through ovipositing. The development of the scale in each County was checked periodically to properly time the parathion sprays. These sprays were well timed in Tulare and Fresno Counties but in Merced County the spray application was delayed until June 4. At this time a few adult female scales could be found on the leaves. A second and later parathion spray was applied in each orchard as a combination Pacific mite and scale

Table 1.—Sprays for control of fig scale in 1949.

TREATMENT NUMBER	TREATMENT PER 100 GALLONS	DATE SPRAYED	PER CENT DEAD ADULT FEMALES[1]	NUMBER SCALES PER LEAF[2]	PER CENT FIGS WITH 4 OR MORE SCALES[3]
	Kadota figs—Tulare County				
1.	Tank-mix dormant oil, 4 gals; blood albumin spreader, 4 ozs.	2-15-49	—	0.9	0.5
2.	Dormant emulsive oil, 4 gals.	2-15-49	—	0.9	0.6
3.	Dormant oil emulsion, 5 gals.	2-16-49	—	0.5	0.2
4.	Parathion 25%, 10 ozs.	5-12-49	—	0.2[4]	0.1
5.	Parathion 25%, 1 lb.	6-24-49	—	—	2.9
6.	Unsprayed check	—	—	47.1	13.9
	Adriatic figs—Merced County—initial infestation 3.6 scales per inch				
7.	Dormant emulsive oil, 3.5 gals.—spray men riding rig.	2-21-49	95.6	13.3	2.8
8.	Dormant emulsive oil, 3.5 gals.	2-21-49	97.9	4.0	0.7
9.	Dormant oil emulsion, 4.5 gals.	2-22-49	98.8	4.7	0.3
10.	Parathion 25%, 15 ozs.	6-4-49	—	2.0[4]	0.03
11.	Parathion 25%, 1 lb.	7-7-49	—	—	0.8
12.	Unsprayed check	—	40.5	103.9	15.1
	Adriatic figs—Fresno County—initial infestation 5.6 scales per inch				
13.	Dormant oil emulsion, 5 gals.	2-17-49	94.7	18.9	11.6
14.	Dormant oil emulsion, 5 gals.; parathion 25%, 10 ozs.	2-17-49	99.1	5.7	1.5
15.	Tank-mix dormant oil, 3 gals.; blood albumin spreader, 4 ozs.	3-11-49	98.8	12.1	10.5
16.	Dormant emulsive oil, 3 gals.	3-11-49	94.1	16.3	14.2
17.	Parathion 25%, 10 ozs.	5-13-49	—	6.0[4]	6.8
18.	Parathion 25%, 1 lb.	7-7-49	—	—	5.4
19.	Unsprayed check A	—	54.1	147.4	55.6
20.	Unsprayed check B	—	—	—	49.6

[1] Number of scales counted per treatment: Merced Co. 686 to 1133, Fresno Co. 1515 to 3181.
[2] Number of leaves examined per treatment: Tulare Co. 288, Merced Co. 96, Fresno Co. 128.
[3] Number of figs counted per treatment: Tulare Co. 2184 to 2399, Merced Co. 3600, Fresno Co. 3200.
[4] Number of live scales. Most scales on the leaves were killed by the spray.

control. A higher dosage of parathion was used in sprays applied after the appearance of adult female scales on the leaves (after June 1).

Unless otherwise noted, all sprays were applied from the ground while the spray men walked around the trees.

RESULTS.—The results of the tests are summarized in table 1. The effectiveness of the sprays was measured in three ways. In the case of winter sprays, the mortality of the adult females was determined one month or more after application. A count of the number of scales per leaf was made at the end of the first summer brood. These counts were made during the period June 20 to July 5—the first counts in Tulare County and the last counts being made in Merced County. Counts of scaly figs were made at harvest. Leaf counts and fruit counts were made in the manner previously described (1949). In general, there was good agreement between the three measures of control in regard to the order of effectiveness of the treatments.

If Kadota figs have more than four scale marks they are thrown out of the canning grade with consequent loss of value. With Adriatic figs, which are packaged as dried figs, there is no uniform agreement among packers on scale tolerance. The packers recognize scaly fruit, however, and show little interest in purchasing such inferior figs. For comparison, data are given on Adriatic fruit with four or more scales per fig.

A statistical analysis was made of the number of figs with four or more scales. The standard errors of the means were employed. In each of the three orchards, treatments were examined by pairs. A summary of the results at the 5 per cent level of significance is as follows:

Tulare County—Kadota figs
- Treatments 1, 2, 3, and 4 better than 5.
- No difference among treatments 1, 2, 3, and 4.
- All treatments better than check.

Merced County—Adriatic figs
- Treatment 10 better than all other treatments.
- Treatments 8, 9, and 10 better than 7.
- No difference among treatments 8, 9, and 11.
- (Difference between treatments 7 and 11 significant at 6% level.)
- All treatments better than check.

Fresno County—Adriatic figs
- Treatment 14 better than all other treatments.
- No difference between treatments 17 and 18 but both better than treatments 13 and 16.
- No difference among 13, 15, and 16.
- All treatments better than either check.
- No difference between checks.

The per cent of figs with 1 to 3 scales closely followed the per cent of figs with 4 or more scales except for the Fresno orchard. In the Tulare County orchard, treatments ranged from 0.4 to 9.8 per cent figs with 1 to 3 scales while the check showed 40.2 per cent figs with 1 to 3 scales per fruit. In the Fresno County orchard, the per cent of figs with 1 to 3 scales ranged from 22.7 to 46.3 among the treatments. The unsprayed checks showed 38.2 and 41.6 per cent figs with 1 to 3 scales.

A comparison of the same treatments in different orchards shows the tendency for more individuals to survive a given treatment in heavier infestations. This tendency was reflected in the per cent of figs with 4 or more scales at harvest. The following comparisons illustrate this point: treatments 3 and 13, 4 and 17, and 11 and 18.

Parathion wettable powders in combination with petroleum oil emulsions are considered to be too hazardous to apply. Neither manufacturers nor governmental agencies advocate the use of parathion with oil.

Figs collected from treatment number 5 in the Merced County orchard on the first day of harvest showed no parathion residue. This was 41 days after spraying.

SUMMARY.—The degree of initial infestation influenced the control by either dormant oil sprays or parathion foliage sprays as measured by per cent of figs with 4 or more scales at harvest.

Dormant oil sprays of the tank-mix type, emulsive type, or emulsion type gave approximately equal control in any one orchard, when used at dosages that gave the same amount of actual oil.

Ten ounces of 25 per cent parathion wettable powder per 100 gallons of water applied in mid-May gave equal or better control than the recommended dormant oil sprays.

In two orchards 1 pound of 25 per cent parathion wettable powder per 100 gallons of water applied in early July gave equal or better control than dormant oil sprays. The same spray applied in late June in another orchard gave less control of fig scale than dormant oil sprays.

After 41 days (July 7 to August 17) no parathion residue was found on figs sprayed with 25 per cent parathion at one pound per 100 gallons of water.

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A Comparison of Methods for Estimating Populations of Spittlebug Nymphs in Alfalfa[1]

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The standard method used by the authors to estimate populations of the meadow spittlebug, *Philaenus leucophthalmus* (L.), in alfalfa has been to count the stems bearing spittle masses in lots of 10 stems collected at random. Observations made in previous years have suggested that the application of certain insecticides reduced not only the number of stems bearing spittle masses but also the number of nymphs per infested stem. In 1949 some additional evidence substantiating these observations was obtained by counting, in both treated and untreated plots in three fields in Wisconsin, (1) the infested stems and (2) the number of nymphs on infested stems. The results of these counts and the per cent control indicated by the two methods are shown in table 1.

The data presented show that the per cent control as calculated from counts of infested and uninfested stems (column 3) is lower than the per cent control figured by multiplying the number of infested stems by the average number of nymphs per infested stem (column 6). The latter figure shows more accurately

[1] This study was supported in part by the Research Committee of the Graduate School of the University of Wisconsin from funds supplied by the Wisconsin Alumni Research Foundation.

Table 1.—Control of meadow spittlebug nymphs with insecticides as indicated by counts of 20 infested stems and by counts of nymphs.

Field Location	Insecticide	By Stem Count			By Nymph Count		
		Number Examined Col. 1	Number Infested Col. 2	Per Cent Control Col. 3	Average Number per Stem Col. 4	Index of Infestation (Col. 2× Col. 4) Col. 5	Per Cent Control (Col. 5) Col. 6
Sheboygan County Farm	Toxaphene	80	2	94.9	1.0	2.0	98.9
	Lindane[1]	80	0	100	1.4	<1.4	>99.2
	Check	80	39	—	4.75	185.2	—
Buboltz Farm	Toxaphene	100	4	87.1	1.35	5.4	95.2
	Check	100	31	—	3.6	111.6	—
Prange Farm	Toxaphene	80	12	79.3	1.75	21	97.2
	Check	80	58	—	13.0	754	—

[1] Although the count in the sample from the lindane plot showed no infested stems, there was a very low infestation (less than 1%), and 20 infested stems were taken to obtain the data in column 4. The figure in column 4 is based on the assumption that one of the 80 stems in column 1 was infested.

the reduction in spittlebug population. However, counting of nymphs is too time-consuming to permit examination of adequate samples in ordinary field work. Counting of infested stems has proved to be a rapid and, in general, a satisfactory method. According to the data presented, the efficiency of tne insecticides would actually be higher than that indicated by the stem-count method. Estimates of efficiency based on such counts should therefore be conservative.

Control of Serpentine Leaf Miner on Pepper

HERMAN S. MAYEUX and GEORGE P. WENE[1]

A serpentine leaf miner believed to be *Liriomyza pusilla* (Meig.)[2] has been a major pest of bell and hot peppers in the Lower Rio Grande Valley, especially in Cameron County, since 1940. The miners infest many cultivated crops in the area but have become a serious problem only on peppers. Some of the cultivated host crops include beans, broccoli, caggage and other cole crops, carrots, cucurbits, celery, lettuce, mustard, okra, onions, English peas, field peas, peppers, potatoes, radishes, spinach, tomatoes, turnips and legumes. Many ornamental and wild plants also serve as hosts of this insect. Heavy infestations on pepper cause defoliation resulting in reduced yield. The insect is active during all seasons of the year. Field observations in 1948 indicated that miners became more numerous following the use of certain poisons, especially benzene hexachloride and DDT.

Wolfenbarger (1947) described leaf miner injury, reviewed the literature, and reported that chlordane as a high volume spray at 1.3 pounds of technical material per acre gave better control in Florida than gamma benzene hexachloride.

A single demonstration-test was conducted on bell pepper in Cameron County, Texas in the fall of 1949 to compare the effectiveness of a dust containing 5 per cent chlordane-5 per cent DDT plus fungicide, with a dust containing 1 per cent gamma benzene hexachloride-5 per cent DDT plus fungicide for the control of leaf miner on bell pepper. The latter dust is used extensively in the area for the control of pepper weevil, *Anthonomus eugenii* Cano, and fruitworm, *Heliothis armigera* (Hbn.). Two replicates of half-acre plots were dusted six times with rotary hand dusters at 20 pounds per acre on a seven-day schedule.

Records were made on October 26, following the third application, of the number of mined leaves on 25 plants per plot. There was an average of 13 mined leaves per plant in the chlordane-DDT plots, 23 per plant in the benzene hexachloride plots and 49 per plant in the untreated.

Table 1 shows the results of examinations of 10 leaves per plant from 25 plants in each plot following the fourth and fifth application and three weeks after the sixth (final) application. The first two records show that the chlordane-DDT dust was twice as effective as the benzene hexachloride-DDT dust. Leaf miners increased very rapidly following the last application of both insecticidal mixtures. Three weeks after the last application mines were as numerous in the benzene hexachloride plots as in the untreated, as shown in table 1.

One month after the last application (December 15) there were so many mines on leaves of all treated plots that it was impossible to count them. Leaves in the treated plots were white with miners and could be distinguished from the greener untreated plots at a distance. By the end of December the treated plots were almost completely defoliated except for a small amount of new growth in the top. Untreated plots were in better condition. Adult leaf miners were observed at the rate of at least five per plant over the entire field on December 15. The destruction of natural enemies by the use of the insecticides and the presence of more tender foliage in treated plots to which flies were attracted are possible explanations for this phenomenon.

[1] Associate County Agent—Entomology, Lower Rio Grande Valley and Entomologist, Substation 15, Weslaco, Texas, respectively. The writers also wish to acknowledge assistance in various ways by Douglas Earley, Port Fertilizer Co. and W. B. Speer, farmer.

[2] Determined by C. W. Sabrosky, Division of Insect Identification, as *Liriomyza sp.* (*pusilla* group).

Table 1.—Results of six applications of various dusts for leaf miner control on bell pepper.

TREATMENT	UNDAMAGED LEAVES	AVERAGE MINES PER LEAF	PER CENT CONTROL
(Following fourth application)			
5% chlordane-5% DDT-fungicide	43%	1.2	80
1% gamma BHC-5% DDT-fungicide	15	3.5	40
Untreated	6	5.9	—
(Following fifth application)			
5% chlordane-5% DDT-fungicide	27	2.7	77
1% gamma BHC-5% DDT-fungicide	7	7.1	34
Untreated	0.4	11.8	—
(Three weeks after sixth application)			
5% chlordane-5% DDT-fungicide	6	21	21
1% gamma BHC-5% DDT-fungicide	0	28	7
Untreated	0	26	—

Pods were counted on 40 plants per plot on November 18, following the sixth application. Average number pods per plant were as follows: chlordane-DDT, 8.6; benzene hexachlorde-DDT, 6.6; untreated, 5.4 pods per plant. On the same date fallen pods were counted on a total row space of 100 yards in each plot (5 areas of 20 yards each). The average number of fallen pods per 100 yards follows: chlordane-DDT, 9.5; benzene hexachloride-DDT, 12.5; untreated, 15. These two records indicate the amount of fruit shedding caused by leaf miner in untreated peppers as compared to partial control by the benzene hexachloride and good control by chlordane. These differences can be attributed to leaf miners as there was very little injury by other insects up to this time.

Very few dead larvae were observed at any time during the test period. Neither benzene hexachloride nor chlordane killed many larvae, if any, inside the mines. Control is attributed to destruction of the adults before eggs are deposited in the leaf tissue.

SUMMARY.—Results of a randomized demonstration-test in the Lower Rio Grande Valley of Texas in 1949 showed that a dust containing 5 per cent chlordane-5 per cent DDT used weekly at 20 pounds per acre gave 77 to 80 per cent control of serpentine leaf miner on bell pepper as long as the schedule of applications was maintained. A dust containing 1 per cent gamma benzene-hexachloride-5 per cent DDT, used similarly, gave only 34 to 40 per cent control. When dusting was discontinued the leaf miners quickly became much more numerous than in untreated areas, defoliating the plants almost completely. The set of fruit on plants and the number of fallen pods was proportional to the degree of leaf miner control.

Results of this test indicate that good control of serpentine leaf miner is possible with a 5 per cent chlordane-DDT dust applied weekly at 20 pounds per acre. Observations indicate that miners are likely to become very heavy when dusting is discontinued or when irregular applications are made. Control of miners with chlordane-DDT dust appears to be due to the destruction of adults before eggs are deposited in the leaf tissue and not by killing larvae in the mines.

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Notes on the Almond Moth

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The almond moth, *Ephestia cautella* Wlkr., is one of several species of phycitid moths of the genus *Ephestia* that attack stored-food products in the United States. It so closely resembles the Mediterranean flour moth, *E. kühniella* Zell., and the tobacco moth, *E. elutella* (Hbn.), that it probably has been frequently confused with them. Described by Francis Walker in 1863 from Ceylon its presence in the United States was first recorded in 1884 when it was found in a shipment of cottonseed cakes consigned from Galveston, Tex., to England. F. H. Chittenden, who wrote several early accounts of its occurrence in the United States, thought that it undoubtedly had been introduced into this country at a much earlier date.

It has been observed from time to time as a pest of nut meats and dried fruit and has been referred to as both the fig moth and the dried currant moth. However, it is doubtful whether its true importance as a pest of foodstuffs and its widespread occurrence in the United States has been fully realized.

An investigation of reported outbreaks of a moth in the warehouses of bean dealers in Texas in 1948 and 1949 revealed that the insect involved was the almond moth. Black-eyed peas in particular were being attacked and seriously damaged. The black-eyed peas, which are fumigated as a routine practice to control field infestation by the cowpea weevil, *Callosobruchus maculatus* (F.), quickly became infested by the moth when the bags were placed in warehouse storage pending packaging or reshipment to other dealers. Although the insect is troublesome chiefly during the summer months, moths were observed flying in the bean warehouses in San Antonio in January 1950.

In a similar study of insect infestation of seed houses in Oklahoma and Texas, this moth was observed to be universally present in seed stores and was particularly troublesome as a pest of seed corn. It was also common in grain storages on farms in the Gulf States, where it was found infesting both rough rice and grain sorghums.

The closely related tobacco moth (*Ephestia elutella*) is reported to be a serious pest of wheat in bulk storage in England. So far this species has not been observed as a pest of stored wheat in the United States. However, in the fall of 1949 a heavy surface infestation of the almond moth was discovered in wheat stored in a ship's hold on the Hudson River. The larvae were migrating in such large numbers that they covered the deck around the hatch openings.

The almond moth is potentially a serious pest of many types of stored food products. It is more likely to be destructive in the southern states and in the Atlantic and the Pacific seaboard states, where the climate is mild, than in the colder parts of the United States.

Although some field infestation of dried fruits, nut meats, and similar products may occur in warm climates, *Ephestia cautella* is essentially a warehouse pest and subject to control by sanitation, good warehouse management, and fumigation. In warehouses that are not adapted for fumigation, pyrethrum sprays are useful in controlling this moth.

DDT-Oil Emulsion for Combined Control of Corn Earworm and European Corn Borer

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Two tests with DDT emulsions containing mineral oil for control of the corn earworm, *Heliothis armigera* (Hbn.), were conducted on Country Gentleman sweet corn at Gibson City, Illinois, in August 1949. During the course of evaluating the control obtained, populations of the European corn borer, *Pyrausta nubilalis* (Hbn.), in the ears were counted. As reported by Ditman (1950), the spraying of sweet corn ears for control of the corn earworm may under some conditions greatly reduce ear infestation by the European corn borer as well as the earworm.

All the emulsions used in these tests were prepared from the same basic formula, but the concentrations of DDT and mineral oil and the number of spray applications were varied. The DDT was in the form of a proprietary 25-per cent emulsifiable concentrate with xylene as the solvent, but the emulsifier was unknown. The mineral oil had a viscosity of 80 to 90 seconds Saybolt.

In each test about 2 ml. of emulsion was sprayed on each ear from a 3-gallon pump-up hand sprayer with a hollow-cone nozzle that would apply approximately 2.5 gallons per hour at 45 to 50 pounds' pressure. In each of three replicated plots, 35 to 40 consecutive ears in a single row were treated, and a similar lot of ears left untreated as a check. Twenty-five ears per plot were examined in the first test and 11 to 25 ears in the second test.

In the first test the first application to plots receiving two and three treatments was made on August 10, when 25 per cent of the ears were in silk. The second applications to these plots and the one-treatment applications were made on August 13, when 76 per cent of the ears were in silk, and the third application was made on August 15, when 96 per cent were in silk.

In the second test the first application was made on August 18, when 30 per cent of the ears were in silk, the second on August 21 at 50 per cent silk, and the third on August 24. The percentage of ears in silk on August 24 was not determined.

The Country Gentleman corn sprayed in these tests was much more susceptible to damage by the DDT-oil emulsion than any hybrid treated in a large number of previous tests. The spray caused shrinkage and separation of the husks and exposure of the kernels to weathering. This exposure also gave the earworm and the corn borer larvae free access to the kernels.

The data are summarized in table 1. In the first test three applications of emulsions without oil and with 5 and 10 per cent of oil gave definitely better results than only two applications of the same materials. One or two applications of the 1-per cent DDT emulsions with 25 per cent of oil and one application with 50, 67 or 75 per cent of oil gave about the same results as three applications of an emulsion containing 0.5 per cent of DDT with 5 per cent of oil or 1 per cent of DDT with 5 and 10 per cent of oil. The highest percentages of worm-free ears and the greatest reductions in numbers of corn earworm

Table 1.—Control of the corn earworm and the European corn borer by hand spraying individual ears of Country Gentlemen sweet corn with various DDT emulsions containing mineral oil.

Test 1					Test 2				
Treatment		Per Cent Worm-free Ears[1]	Per Cent Reduction in Larvae		Treatment		Per Cent Worm-Free Ears	Per Cent Reduction in Larvae	
Per Cent of DDT	Per Cent of Oil		Earworm	Borer	Per Cent of DDT	Per Cent of Oil		Earworm	Borer
		Two Applications					Two Applications		
0.5	5	60	72	86	0.25	5	34	35	34
1	0	51	61	63		10	26	28	25
	5	72	85	73	0.5	5	35	20	47
	10	79	84	78		10	33	49	52
	25	88	93	97	1.0	0	17	14	45
		Three Applications				5	44	55	68
0.5	5	87	93	95		10	37	45	62
1.	0	77	86	85			Three Applications		
	5	85	92	93	0.25	5	42	54	52
	10	99	99.6	93		10	53	51	55
		One Application			0.5	5	55	70	81
1.	25	89	94	94		10	67	80	65
	33	80	89	80	1.	0	59	57	71
	50	92	95	97		5	60	77	84
	67	95	96	96		10	75	78	86
	75	89	90	96			Untreated Check		
		Untreated Check						*Number per Ear*	
			Number per Ear				3	2.51	1.61
		4	2.23	1.96					

[1] Ears having neither living earworms nor earworm damage.

and corn borer larvae per ear were obtained in plots where an emulsion with 1-per cent of DDT and 10 per cent of mineral oil was applied three times and also where a 1-per cent DDT emulsion containing 50 or 67 per cent of mineral oil was applied only once. Unfilled and damaged ears were noticed in plots sprayed with emulsions containing DDT and 25 to 75 per cent of mineral oil, although it is thought that this was due, at least partially, to the high susceptibility of the Country Gentlemen hybrid to spray damage.

In the second test, the spray caused greater husk separation and ear damage than in the first test. In all trials three applications showed a definite increase in worm-free ears as well as in reduction of the earworm and borer larvae, as compared with two applications. The three applications of 0.5 and 1-per cent DDT emulsions containing 10 per cent of mineral oil gave the most worm-free ears. The greatest reduction in numbers of earworms occurred in plots treated three times with emulsions containing 0.5 or 1 per cent of DDT with 10 per cent of mineral oil or 1 per cent of DDT with 5 per cent of oil. The greatest reductions in numbers of corn borers occurred in plots receiving three applications of emulsions with 0.5 or 1 per cent of DDT plus 5 per cent of oil, or an emulsion containing 1 per cent of DDT with 10 per cent of oil. The lack of proper timing of the spray applications undoubtedly contributed to the poor control in this test.

The results obtained from these preliminary tests indicate the possibility of obtaining good control of both the earworm and the borer in sweet corn by spraying the ears with a DDT emulsion containing mineral oil at the proper time.

LITERATURE CITED

Ditman, L. P. 1950. Control of corn earworm and other pests in corn. JOUR. ECON. ENT. **43**(2): 191–3.

A Food Supplement for Effectively Increasing the Fecundity of Certain Tephritid Species

KENNETH S. HAGEN[1] and GLENN L. FINNEY,[2]
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A commercial enzymatic protein hydrolysate of yeast[1] containing free amino acids, polypeptides, all factors of the Vitamin B complex found in yeast, and a total protein content of 70 per cent (N×6.25) was found to produce high fecundity in the females of *Chrysopa californica* Coquillett when present in their diet (Hagen 1950).

Enzymatic yeast hydrolysates[1,2] are also being used successfully in the mass-culturing of *Dacus dorsalis* Hendel, *D. cucurbitae* Coquillett & *Ceratitis capitata* (Wiedemann). Not only does the yeast hydrolysate when ingested shorten the preoviposition period as compared to diets containing powdered brewer's yeast, but it increases fecundity as reflected in the consistent high level of egg deposition obtained throughout the life of the flies.

A yeast hydrolysate diet alone is not adequate since there is a lack of carbohydrate. Either honey or sucrose can be utilized as the carbohydrate source.

An effective combination found thus far consists of a 20 to 50 per cent yeast hydrolysate solution smeared on wax paper accompanied with another sheet of wax paper brushed with honey or an 80 per cent sucrose solution. Water should be supplied separately. Solid yeast hydrolysate with solid sucrose is also an effective diet for both *Dacus* species.

LITERATURE CITED

Hagen, Kenneth S. 1950. Fecundity of *Chrysopa californica* Coquillett as affected by synthetic foods. JOUR. ECON. ENT. **43**(1): 101–4.

[1] Principal Laboratory Technician in the Experiment Station.
[2] Assistant Specialist in the Experiment Station.
[1] Protein Hydrolysate—MRT. Marvin R. Thompson, Inc., Stamford, Conn.
[2] Yeast Hydrolysate (Enzymatic), Nutritional Biochemicals Corp., Cleveland, Ohio.

A Method of Rearing the Flat Grain Beetle and the Grain Mite

ALBERT C. APT, *U.S.D.A., Agr. Res. Adm. Bureau of Entomology and Plant Quarantine*

The flat grain beetle, *Laemopholoeus minutus* (Oliv), and the grain mite, *Acarus siro* (L.), are often found abundantly infesting accumulations of damp stock at the bottom of elevator boots in flour mills throughout the North Central States. The presence of the grain mite in large numbers is a good indication that the product in which it occurs is high in moisture content.

In the writer's experiments at Minneapolis, Minnesota, it was necessary to maintain healthy cultures of these two pests. To keep the culture medium at the high moisture content required for rearing the flat grain beetle successfully the following procedure was developed.

A cardboard ice-cream carton 11.5 inches in height and 4½ inches in diameter was half filled with patent flour and artificially infested with flat grain beetles. A glass vial 3.75 inches in height and 0.75 inches in diameter was inserted to one-half of its length into the flour and kept full of water. The carton was covered and placed in a dark cabinet, where the temperature was maintained at about 80° F. A healthy culture has been maintained for more than 4 months and shows every indication of continuing indefinitely.

Practically the same method is being used in rearing the grain mite, the only difference being that a half-pint wide-mouthed screw-top glass jar is used as the container. A healthy culture has been maintained for more than 5 months and also shows indications of continuing indefinitely.

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Extension Entomology[1]

During the last half of the nineteenth century a number of insect pests, such as San Jose Scale, boll weevil, and Colorado potato beetle, began to ravage this country. By 1914 the boll weevil was so destructive that the need for prompt nation-wide action did much to bring about the passage of the Smith-Lever Act, which provided for cooperative participation between the federal government and all of the states, and the establishment of the Cooperative Extension Service.

The growing importance of insect pest problems has constantly increased the need for extension work in entomology. Additional insects, many of foreign origin, such as the European corn borer, the oriental fruit moth and the Japanese beetle have caused serious damage in this country. The European corn borer which has now spread over the main corn belt, caused an estimated loss of $349,000,000 in 1949. This amount is far in excess of that spent for research and control of insects by our state and federal governments. The oriental fruit moth has now spread to all major fruit growing areas in the United States and a constant battle needs to be waged to prevent its destructiveness. The Japanese beetle is in most of the states east of the Mississippi River. It is doubly destructive because the grubs feed on the roots and the beetles on the foliage, flowers and fruits of so many different kinds of plants. The Mediterranean fruit fly and the citrus black fly became established in Florida and the date scale in the southwest. Apparently intensive efforts to eradicate these three insects were successful.

At the present time numerous other important insect pests on foreign shores gaze hungrily at our fields and orchards. But the insects themselves are not our only problems. The benefits of science and research have deluged us with a number and variety of insecticides that leave the layman bewildered and confused. The recent edition of *Entoma* lists at least 50 basic chemicals and about 2500 trade-name products. Which one of these to use for which insect, on which crop, in what strength, how toxic is it, what flavor does it add or destroy, how can the residues be removed, and what is the danger to men, animals, and equipment? These are but samples of the questions that an increasing number of farm and city people are asking extension entomologists. We, as well as those who are engaged in research, regulatory, control, or commercial work, have a responsibility in supplying the public with all possible answers to these questions.

The function of the Extension Service is educational, producing changes in human behavior. Congress had this in mind when it drafted the Smith-Lever law in 1914. The law states, in part, that the object of the Extension Service is "to aid in diffusing among the people of the United States useful and practical information on subjects relating to agriculture and home economics, and to encourage the application of the same . . . through field demonstrations, publications, and otherwise."

You have heard of the Extension Service for years and many of you have been extension workers. Therefore, it should suffice to state that the Federal Office of the Extension Service consists of six divisions: Administration, Information, Field Coordination, Field Studies and Training, Agricultural Economics and the Division of Subject Matter. A somewhat similar pattern is followed in some of the states.

The Divisions of Subject Matter and Agricultural Economics are made up of professional people who are closely associated with their respective research and service bureaus or agencies.

The professional people in this group serve as liaison between the old-line research bureaus, the action agencies, and the Extension Service. They also form a

[1] Presented at the A.A.E.E., Pacific Slope Branch, June 14, 1950.

two-way bridge between state research and extension and federal research and extension. There are 42 specialists on the federal staff, including one entomologist.

The Extension Service has been referred to as the educational arm of the Department of Agriculture and possibly the greatest institution for adult education in the world. The statement can be justified when we consider that the Extension Service consists of about 12,000 people trained in the subjects of agriculture and home economics, as well as the necessary clerical help. Considering that in practically every one of the 3000 counties there are from 1 to 12 county extension agents the educational position of extension is all the more obvious.

Of the 12,000 people (exclusive of clerical) in the Extension Service about 9000 are in the counties, 2700 on the state level and less than 100 in the federal office.

In 1949 funds in the amount of $68,487,000 were necessary to support the Extension Service. The strength of the service may be attributed, in part, to the fact that all funds do not come from the federal government. About 46 per cent of the extension funds come from federal sources, 28 per cent from state and about 26 per cent from within the counties. The pattern of expenditures is quite different. Less than 2 per cent is spent in the federal office, 32 per cent in the state office and 66 per cent is spent in the counties.

Of the 32 per cent spent on the state level about 20 per cent is spent for specialists' work. This includes all of the 1933 men and women specialists, 65 of whom are entomologists.

Extension workers operate under the terms of a general memorandum of understanding between the presidents of the land-grant colleges and the Secretary of Agriculture, U. S. Department of Agriculture. Then there is a project agreement for each major line of subject matter, which outlines the work of the specialists. Each project is signed by a State Extension Director and the Federal Extension Director. For each subject-matter project the specialists prepare an annual plan of work and an annual report for the use of the specialists and others in the state and federal offices.

All states except Maine, New Hampshire, Vermont, Connecticut, Georgia, Florida, Kentucky, South Dakota, and Nevada have entomologists working under an extension project. Pennsylvania still leads the list with the greatest number of full time extension entomologists, six men, one of whom spends full time on extension apiculture. This does not imply that they are overstaffed but are a little better equipped to meet their needs.

The need in the Extension Service for more trained entomologists is a result of the increasingly serious insect pest control situation. The ultimate object of these specialists is to help reduce the losses caused by insects and to protect the health of man and animals by controlling the destruction, the disease carrying and the pestiferous insects. The extension entomologist has to do this through stimulating the public to take the necessary action. His efforts must be more than mere trouble shooting. Many methods and avenues of approach must be used to do the job. As extension entomologists, our first responsibility is to fortify the more than 9000 county extension agents with up-to-date subject-matter information and assist them in getting the public to adopt it.

We reach the agents in many ways. The most effective is a visit to the county when the agent is in the midst of the insect problem and the subject is uppermost in his mind. In addition to providing the agent with subject-matter information, we assist him in setting up method and result demonstrations, in conducting farm tours or caravans, and in the preparation of visual informational materials, such as exhibits, models, mounted specimens, color slides, motion pictures, chalk talks, flannelgraphs, and sketches for circular letters. The specialist also helps the agent in the preparation of spot-news stories and radio releases based on timely information and local conditions. The specialist is sometimes criticized when he goes into a county with plenty of equipment and puts on a good show, then picks up his wares and moves to another county, leaving the agent with no illustrative material to use, and wonders why the agent does not carry on the good work the specialist started. The difficulty has been overcome in many states where the specialist has cooperated with informational people in the preparation of ex-

hibits, slide series, and motion pictures that can be lent to county agents. It is too much to expect that there will ever be enough extension entomologists to adequately serve the agents through visits to the county. An effective substitute has been to bring about half a dozen agents together at some central point for a refresher course in entomology. It has been found that the response is better and the agents get more from such conferences if the number of agents is kept small. One or two such training schools a year are not adequate to fortify the agents to meet all situations, because the insect picture is constantly changing. Then, too, new insecticides are constantly coming on the market. A number of extension entomologists are keeping the agents informed by issuing weekly newsletters reporting the occurrence, abundance, and development of the different insect pests and giving information about control. In some states such letters are issued at irregular intervals as the necessity arises. Sometimes a joint letter is issued by the entomologist and plant pathologist. In some cases the entomological material assembled by the extension information people is combined with similar material from the other specialists, and then sent to the counties as a packet. Naturally the entomological material is not observed and used by the agent as readily when received in a packet. Radio releases are prepared in the state office and distributed to the county agents. Most press and radio releases are prepared cooperatively with the extension editorial office.

Many press and radio releases are prepared in the Bureau of Entomology and Plant Quarantine. Copies of these are mailed to the states, where they are used as received or are localized and then used.

A few years ago a survey was made of releases issued by the states, and more than half of those examined were traceable to releases issued by the Bureau of Entomology and Plant Quarantine. To give the articles in the press greater appeal some states have established a mat service. Some extension entomologists issue loose-leaf handbooks for county agents' use. In this way new material can be sent to the agents when it is needed and the agents can readily replace the old with the new.

The specialist also prepares bulletins, circulars, leaflets and other forms of subject-matter material for printing or duplicating. Naturally he cooperates with subject-matter specialists in related fields so that his recommendations will fit into approved agronomic and livestock practices and will be supported by the other specialists.

The field of entomology is becoming so involved that the specialist and county workers cannot do the job merely by working direct with farmers. They must work with others who have contacts with farmers. In the past few years there has been a big expansion in the commercial application of insecticides. This has brought many air pilots and operators into the field, a number of whom are unfamiliar with insect control. Training schools or refresher courses for these people have been conducted in many states. Most of such operators have appreciated the opportunity to get authentic information that will make their insecticide applications more effective.

The great number of new insecticides makes it difficult for insecticide dealers to keep abreast of the times. This has necessitated closer working relations between the entomology specialists and the commercial people. In many states training schools or conferences have been held and the recommendations for the particular state carefully considered. In this way the dealer has a better understanding of what the recommendations for the state will be. With a better knowledge of insecticides he will be more likely to carry in stock those that are recommended. In a few states an annual meeting is held between extension and research entomologists and plant pathologists, and a rather select representation of industry. The insect and disease control recommendations prepared by the state people are discussed freely and openly by industry and state people. As a result of such a meeting, each person present has a better understanding of the recommendations and the reason for them.

In some states definite organizations of the people having to do with pest control have been set up; for instance, the Ohio Pesticide Institute and the Michigan Insecticide-Fungicide Institute. Dealers, operators, and entomologists with the uni-

versities, make up the membership of these organizations. It is my understanding that these differ somewhat from your Pacific Insecticide Institute. Since the war the GI instructors have been effective in helping to disseminate entomological information, especially in aiding their students in recognizing the more common insect pests and in determining the population levels of insects necessary to warrant the application of control measures.

For many years the extension entomologists have been cooperating with the fieldmen employed by canning companies or other commercial organizations. However, another kind of fieldman has come into the picture in recent years. This is a man employed by farmers to scout their fields to determine the levels of population of certain insect pests. For instance, last year in Louisiana, farmers in 9 parishes employed 50 scouts who surveyed 95,000 acres of cotton. In some cases the infestation for each field was recorded on a map of the farm, one copy of the map was left with the farmer, and another copy was sent to the county agent. It was reported that more scouts would have been employed and more cotton would have been surveyed had competent men been available. Close working relations must exist between the extension entomologists and such field men.

The commodity approach is often used to bring the problem of insect control to a greater number of people. The situation with respect to cotton insects might be cited as an example. Ten years ago the six extension entomologists in the Cotton States were struggling along trying to get the insect-control measures adopted, but progress was slow. About that time a nonprofit organization interested in all phases of cotton production, marketing, and uses was formed. This agency sensed the losses caused by cotton insects and began to arouse the interest of commercial people having to do with cotton. The development of new insecticides contributed much to the program. During the past 3 years federal and state entomologists, from the Cotton States, met at different central points each year. They reported on research and extension experiences and arrived at a general agreement on recommendations, which were published. After each of the three annual regional meetings of entomologists, the organization sponsored public meetings where representatives of all phases of cotton insect control were present. This has done much to bring all agencies interested in the problem together and has inspired them to present a common front against cotton insects. Instead of six extension entomologists in the Cotton States there are now 16. They have the full support of most of the agencies relating to cotton production. In some states all the agencies relating to certain problems have banded together. They formulated programs of work outlining the function of each agency. There are many advantages from such cooperative effort. All are telling the same story about the pests involved and there is a better understanding of control measures. The dealers are more likely to handle the insecticides recommended by the entomologists and, since everyone has a definite part in the program, much more will be accomplished. This has happened in cotton insect control and in the control of houseflies, mosquitoes, the European corn borer, and the grasshoppers.

It would be possible to continue at great length citing examples of the excellent work that the extension entomologists are doing, but the few cases referred to should give briefly the nature of our work.

From the foregoing comments it is obvious that the appointment of the 65 extension entomologists has not excluded all other entomologists from doing extension work. There is plenty of work in entomology for us all to do and the more we cooperate and pull together just that much more effective will be our programs and the stronger will be our profession.

The extension entomologist is only one cog in the wheel. His appointment grew out of the necessity of having someone in the Extension Service whose training and close association with other members of his profession would equip him to better serve other extension personnel and through them the public.

During my quarter of a century in the work it has been gratifying to note how the other members of our profession have accepted us in extension as a very necessary part of the field of Entomology.

National Science Register

A new National Science Register is required by the law establishing the National Science Foundation, but it seems probable that such a register will be needed before the NSF can begin to function. Accordingly, the National Security Resources Board has set up the machinery under the administration of the Office of Education, Federal Security Agency to register the scientific personnel of the nation. James C. O'Brien, of the Office of Manpower has been named Director of the Register.

The National Academy of Sciences-National Research Council has been asked to take the responsibility for certain aspects, including the initial circularization and the development of check lists for sciences. Questionnaires will go out about December first and must be completed and returned at once. The questionnaires will be analyzed by special coding procedures and recorded on punch cards.

The American Institute of Biological Sciences has offered its facilities and the registration of scientists in biology and agriculture has been assigned to that Institute. Physics, chemistry, mathematics, geology, geophysics, meteorology, astronomy, mathematics, and crystallography will be registered in this initial effort by other institutes and national organizations.

The American Institute of Biological Sciences has asked for the cooperation of entomologists and the American Association of Economic Entomologists is furnishing an addressograph tape of all members.

Some of you will recall that at the inception of World War II, address cards were prepared for the National Research Council. This Association organized a committee of three that served as the liaison between the medical departments of the Army and Navy and entomologists desirous of entering the services. Of the more than 700 entomologists who served in the armed forces, over half functioned as entomologists. Since that time, entomologists have come to have a recognized place in the armed forces and are included in tables of organization. It is, therefore, especially urgent that every entomologist return his completed questionnaire promptly.

Foreign Members

Following is a list of names of foreign members whose names are to be integrated with the next general roster. They were omitted inadvertently and are published herewith with apologies. It has been difficult to obtain reliable information in regard to many foreign members although such sources as their embassies in this country, the State Department, and many foreign correspondents have been consulted:

Aullo Costilla, Manuel, Colle Velasquez 80, Madrid, Spain.

Brain, Charles K., 61 Albert St., Waterkloof, Pretoria, South Africa.

Harukawa, Shukichi, Dean, Dept. of Agr., Okayama Univ., Okayama, Japan.

Jack, Rupert W., Umfeleni, Goromonzi, P. O. Arcturus, S. Rhodesia.

Johnston, T. Harvey, Univ. of Brisbane, Queensland, Australia.

LePelly, Richard H., Dept. of Agr., Nairobi, Kenya Colony and Protectorate, Africa.

Lounsbury, C. P., 795 Church Street, East, Pretoria, South Africa.

Mally, C. W., Ohiowa, Victoria Road, Stellenbosch, C. P., South Africa.

Ripper, Walter E., Pest Control Ltd., Harston, Cambridge, England.

Ruszkowski, J. W., Univ. of Wroclaw, Pulawy, Poland.

Stellwaag, Friedrich, Institut fuer Pflanzenkrankheiten, Geisenheim am Rhein, Germany.

Schoyen, W. M., State Entomologist, Statens Plantevern, Zool. Museum, Oslo, Norway.

Troubelot, Bernard, Institut National de le Recherche Agronomique, Route de Saint Cyr, Versailles, France.

Wille, Dr. Johannes, Liberatadores 705, San Isidrio, Lima, Peru.

Yuasa, Hachiro, Pres. Japan Int. Christian Univ., 61 Nakagawara Machi, Shimo, Gamo, Saky-Ku, Kyoto, Japan.

Zander, Enoch, 7 Rathsberger Strasse, Erlangen, Germany.